PLANT PHYSIOLOGY

EDITORS

CHARLES A. SHULL
WALTER F. LOEHWING
JAMES P. BENNETT — CHARLES B. LIPMAN
GEORGE W. SCARTH — MAX A. MCCALL

EDWIN C. MILLER
HENRY R. KRAYBILL — BURTON E. LIVINGSTON

VOLUME 14

1939

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

TABLE OF CONTENTS

Number 1. January

Thomas Andrew Knight: in Memoriam.
Charles A. Shull and J. Fisher Stanfield 1–8
Antagonism of certain elements essential to plants toward chemically related toxic elements. Annie M. Hurd-Karrer 9–29
Rôle of nitrogen in fungous thermogenesis.
John O. Gaskill and Joseph C. Gilman 31–53
Translocation of carbohydrates in the sugar beet. O. A. Leonard 55–74
Foliar diagnosis: the influence of the soil on the action of fertilizers. Walter Thomas and Warren B. Mack 75–92
The relation of cabbage hardiness to bound water, unfrozen water, and cell contraction when frozen. J. Levitt 93–112
Effect of potash manuring on the production of organic matter.
Oscar Eckstein 113–128
Estimation of protoplasmic permeability from plasmolytic tests.
G. W. Scarth 129–143
Effect of ethylene on certain chemical changes associated with the ripening of pears. Elmer Hansen 145–161
Growth behavior of one-millimeter excised root tips.
Gladys C. Galligar 163–169
Transpiration and the absorption of mineral salts.
Kenneth E. Wright 171–174
Time and temperature of protoplasmic coagulation.
Henry T. Northen and Rebecca Tyson Northen 175–176
A method for the treatment of cuttings and roots of the pecan with root-inducing chemicals. C. L. Smith and L. D. Romberg 177–178
Plant physiology in the women's colleges. Dorothy Day 179–181
Notes 183–190

Number 2. April

Daniel Trembley MacDougal, pioneer plant physiologist.
George T. Moore, C. Stuart Gager, and Forrest Shreve 191–202
Carbohydrate and respiratory metabolism in the isolated starving leaf of wheat. G. Krotkov 203–226
Distribution of different nitrogen fractions, sugars and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts.
C. P. Sideris, B. H. Krauss, and H. Y. Young 227–254

62897

580.5
P694
v.14

Effect of temperature upon the rate of elongation of the stems of asparagus grown under field conditions.
C. W. CULPEPPER, and H. H. MOON 255–270
Problems in the storage of cut carnations ..... M. S. NEFF 271–284
Application of the ceric sulphate method in the analysis of carbohydrates in the roots of Lepidium and Convolvulus.
C. G. BARR 285–296
Effect of heavy and late applications of nitrogenous fertilizer on the cold resistance of Kentucky bluegrass.
J. C. CARROLL and F. A. WELTON 297–308
Suggestions for the use of Warburg respirometers in plant physiological investigations ..... JAMES W. BROWN 309–320
Relation between fruit size and food supply in the tomato.
FELIX G. GUSTAFSON and HELEN B. HOUGHTALING 321–332
Organic acid metabolism of the buckwheat plant.
GEORGE W. PUCHER, ALFRED J. WAKEMAN, and HUBERT BRADFORD VICKERY 333–340
Determination of ammonia and amide nitrogen in connection with the chlorate method for nitrogen in plant tissues.
E. M. EMMERT 341–349
Determination of constants for curves of water absorption by dry organic substances ..... CHARLES A. SHULL and S. P. SHULL 351–357
Electrical response of *Phaseolus multiflorus* to electrical currents.
W. S. REHM 359–363
Transport of inorganic ions in polar plant tissues ..... F. W. WENT 365–369
The essentiality of certain elements in minute quantity for plants with special reference to copper.
D. I. ARNON and P. R. STOUT 371–375
Alkaloids from *Sanguinaria canadensis* and their influence on growth of *Phymatotrichum omnivorum*.
GLENN A. GREATHOUSE 377–380
Seasonal sugar variations in alfalfa ..... J. C. IRELAND 381–384
Relations of plants to minute doses of inhibitive substances.
ERNEST S. REYNOLDS 385–387
Condition of chlorophyll in the leaf.
O. L. INMAN and MARIE L. CROWELL 388–390
An automatic plant irrigator and recorder.
C. N. JOHNSTON and O. A. ATKINS 391–393
Notes 395–400

NUMBER 3. JULY

Atmospheric humidity and temperature in relation to the water system of plants and soils ..... CHARLES A. SHULL 401–422

Studies on antagonistic phenomena and cation absorption in tobacco in the presence and absence of manganese and boron.
T. R. SWANBACK 423–446
Transpiration and physico-chemical properties of leaves as related to drought resistance in loblolly pine and shortleaf pine.
C. S. SCHOPMEYER 447–462
Some investigations on the assimilation of apple leaves.
J. G. WAUGH 463–477
Influence of certain amino acids and of nicotinic acid upon the nicotine content of tobacco leaves RAY F. DAWSON 479–491
Growth of wheat plants from dry and soaked irradiated grains.
EDNA LOUISE JOHNSON 493–504
Greenhouse studies of the effect of clipping the tops of alfalfa at various heights on the production of roots, reserve carbohydrates, and top growth C. M. HARRISON 505–516
Equipment for the growing of plants at controlled temperatures.
E. MARION BROWN 517–526
Glycine in the nutrition of excised tomato roots PHILIP R. WHITE 527–538
Effects of cations and anions on protoplasmic elasticity.
HENRY T. NORTHEN and REBECCA T. NORTHEN 539–547
Characteristics of the tyrosinase system in potatoes which blacken after boiling.
A. FRANK ROSS, W. E. TOTTINGHAM, and RUDOLPH NAGY 549–557
Carbohydrates of wheat leaves G. KROTKOV 559–565
Stimulation of germination of dormant lettuce seed by sulphur compounds ROSS C. THOMPSON and WILLIAM F. KOSAR 567–573
Some factors contributing to tomato puffing.
J. J. TAUBENHAUS and G. E. ALTSTATT 575–581
Electrodialysis of pea seeds WENDELL R. MULLISON 583–587
Interpretation of comparative growths of fungal colonies growing on different solid substrata CLAIR L. WORLEY 589–593
The calibration of a constant recording illuminometer when the sensitive sampling surface is horizontal.
DONALD R. WILLARD and JOHN B. SMITH 595–598
Molybdenum as an essential element for higher plants.
D. I. ARNON and P. R. STOUT 599–602
Sintered pyrex glass aerators GEORGE H. CARROLL 603–605
Nitrogen nutrition in relation to yield and quality of grapefruit.
WILLIAM E. MARTIN 606–607
Notes 609–614

NUMBER 4. OCTOBER

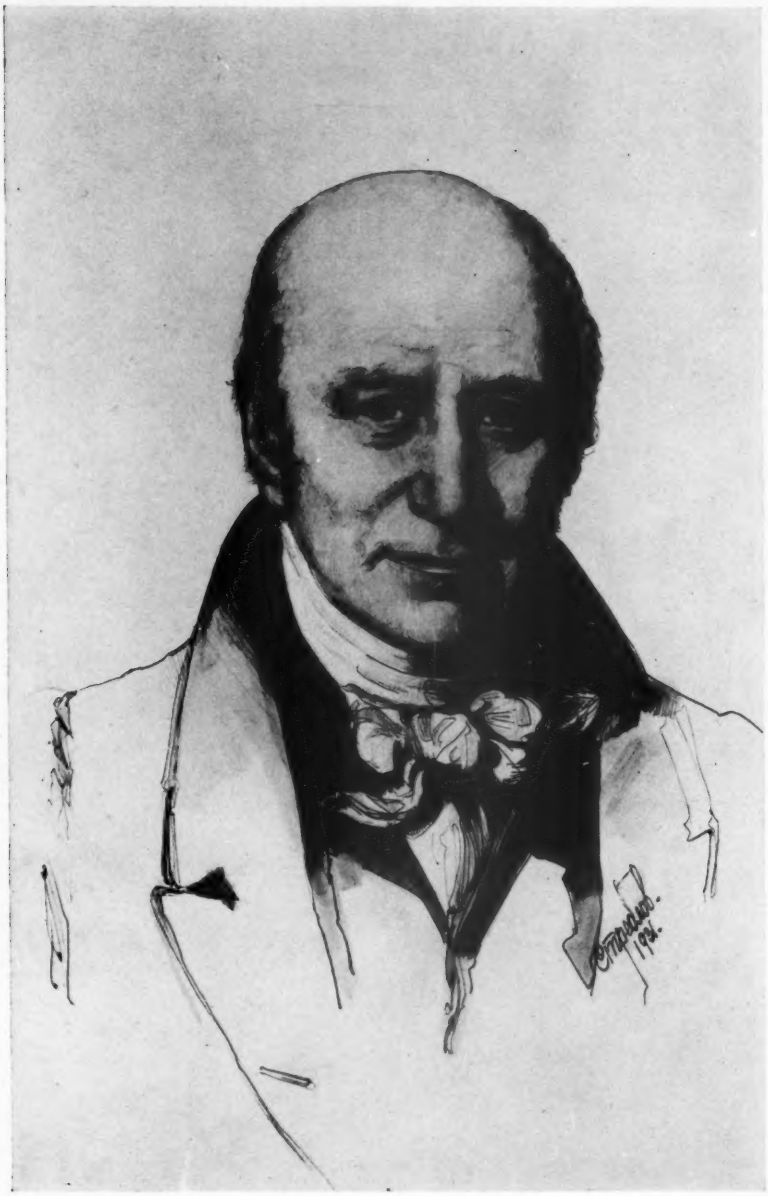
HENRY HORATIO DIXON 615–619
Respiration of acorns as related to temperature and after-ripening.
JAMES W. BROWN 621–645

Distribution of nitrogenous fractions, sugars, and other substances in bananas grown in darkness versus daylight.
C. P. SIDERIS, B. H. KRAUSS, and H. Y. YOUNG 647–676
Changes in the composition and rate of growth along the developing stem of asparagus ..... C. W. CULPEPPER and H. H. MOON 677–698
Foliar diagnosis: physiological balance between the bases lime, magnesia, and potash ..... WALTER THOMAS and WARREN B. MACK 699–715
Effects of certain insecticides and inert materials upon the transpiration rate of bean plants ..... E. C. WAGNER 717–735
Polysaccharides of the vegetative tissues of maize ..... C. G. BARR 737–753
A study of developmental changes in composition of the macadamia ..... WINSTON W. JONES 755–768
The effect of host nutrition on concentration of tobacco-mosaic virus ..... E. L. SPENCER 769–782
Floral development of certain species as influenced by x-radiation of buds ..... EDNA LOUISE JOHNSON 783–795
Some effects of metal salts upon yeast dehydrogenase.
CHARLES WILLIAM HOCK 797–807
Pectic content of plant materials.
WILLIAM E. ELWELL and WILLIAM M. DEHN 809–816
Production and consumption of ethylene by ethylene treated bananas ..... R. C. NELSON 817–822
Evidence for the essentiality of silicon for growth of the beet plant ..... J. G. RALEIGH 823–828
A metering pump for small volumes of solution ..... M. A. RAINES 829–831
Metering pump and turntable arrangement for supplying measured quantities of nutrient solution successively to any number of culture vessels ..... H. G. DU BUY 832–835
Convenient seedling support for growing plants in water culture.
STUART DUNN 836–837
Notes 839–844
General index 845–858

ERRATA

VOLUME 14

Page 5, line 21, delete comma after word wheels.
Page 92, citation 7, first line, for "The" read its.
Page 139, line 9, for "necessary alternatively" read necessary. Alternatively.
Page 142, line 21, for "equation (9)" read equation (19).
Page 187, line 14, for "EDWARD" read EDWIN.
Page 280, last line of legend, for "gallon" read gallon can.
Page 418, line 20, for "is" read it.
Page 445, line 4 of summary, for "effect" read effects.
Page 459, line 13, for "were" read was.



THOMAS ANDREW KNIGHT
AUGUST 12, 1759—MAY 11, 1838

Vol. 14 No. 1

PLANT PHYSIOLOGY

JANUARY, 1939

THOMAS ANDREW KNIGHT
IN MEMORIAM

CHARLES A. SHULL AND J. FISHER STANFIELD

(WITH ONE PLATE[1] AND ONE FIGURE)

During the latter part of the eighteenth century the physiological behavior of plants challenged the attention of many investigators. The brilliant discoveries of PRIESTLY, INGEN HOUSZ, SENEBIER, and DE SAUSSURE were great historical events, and their published works form an impressive and unforgettable chapter in the history of plant physiology. To this galaxy of stellar performers must be added the name of THOMAS ANDREW KNIGHT, who, beginning his investigations during the decade from 1780 to 1790, became subsequently one of the keenest and most prolific students of plant physiology and horticulture. His publications in the *Philosophical Transactions of the Royal Society of London* began in 1795, and more than 20 papers appeared in the *Transactions* up to the year 1828. In addition he published well over 100 papers in horticulture, and several well known independent treatises. His career was brought to a close by death in 1838, at the age of 79 years.

Because he lived during a reactionary period, his work has never been fully appreciated. It seems most fitting, therefore, with the passage of a century since his death, to look back through the years with appreciation and understanding, especially understanding of the primitive state of knowledge throughout the realm of science, to appraise his work, and to commemorate his services to the science which he loved and his great practical services to mankind. His name will forever be associated with, and his work a part of, the history of biological science during the early decades of the nineteenth century.

[1] The plate has been reproduced from a photograph of an original in the private portrait collection of Dr. R. B. Harvey of the University of Minnesota. We wish to express to him our thanks and appreciation for the privilege of reproducing this fine portrait.

The first of his many contributions was entitled *Observations on the Grafting of Trees,* which was written at Elton, Herefordshire, April 13, 1795, as a letter to his friend and adviser, SIR JOSEPH BANKS, who read the letter before the Royal Society on April 30, 1795. The paper discussed the inheritance of decay among fruit trees, and the propagation of debility by grafting.

Encouraged by the reported reception of this paper, he continued, even long after he had been elected a Fellow of the Royal Society, to send his papers to Sir JOSEPH to be read. He was innately shy and modest, often apologetic for his shortcomings in "not being very deeply read in the experiments which naturalists have made on plants" and this may account for the presentation of his reports as letters to his intimate friend.

In appraising his work at the annual meeting of the Royal Society following his death, His Royal Highness the DUKE of SUSSEX, speaking as retiring president, rated the first paper published in 1795 as one of the most valuable of his contributions. This rating was based, no doubt, upon its practical aspects, because it dealt with the problem of "canker and moss," and the propagation of these diseases through grafts or layers to new trees. Plant physiologists, however, attach greater significance to his more scientific studies of gravitational and centrifugal action upon the roots and tops of plants. Many of his papers contain very valuable observations which one wishes were the possession of every student of plant physiology. Lord SUSSEX said: "Mr. Knight was a person of great activity of body and mind and of singular perseverance and energy in the pursuit of his favorite science: he was a very kind and agreeable writer, and it *would be difficult to name any other contemporary author in this or other countries who has made such important additions to our knowledge of horticulture and the economy of vegetation.*" (Italics ours.)

An examination of his papers reveals the truth of the president's estimate. The second paper, *An Account of Some Experiments on the Fecundation of Vegetables,* published in the Transactions in 1799, contains an account of some experiments on peas which he began in 1787. Interested mainly in the improvement of apples, he realized that results would be slow with perennial and slow-to-fruit varieties; he therefore decided to use annuals to obtain information more rapidly as to the effects of fertilization. Speaking of annuals he says: "Amongst these, none appeared so well calculated to answer the purpose as the common pea; not only because I could obtain many varieties of this plant of different forms, sizes, and colours; but also, because the structure of its blossoms, by preventing the ingress of insects and adventitious farina, has rendered its varieties remarkably permanent."

He records the use of the farina (pollen) of a gray pea in fertilizing a white variety. The seeds resulting from the original fertilization were not modified in appearance, but when these F_1 seeds were planted, all of the offspring produced plants with nothing but dark gray seeds. Then he says: "By introducing the farina of another white variety (or, in some instances by simple culture) I found this colour was easily discharged, and a numerous variety of new kinds produced, many of which were, in size, and in every other respect, much superior to the original white kind, and grew with excessive luxuriance, some of them attaining a height of more than twelve feet." Here we see KNIGHT observing dominance, recessive behavior, and heterosis almost 80 years before MENDEL's day. With a more complete understanding, and quantitative analysis of his results in succeeding generations, KNIGHT might easily have occupied the position in genetics now given to MENDEL. One wonders how aware MENDEL may have been of this interesting work carried on by KNIGHT nearly a century earlier; for KNIGHT had the virtue not to place his work in an obscure journal.

He turned his attention to ascent and descent of sap in trees, and showed himself a true disciple of HALES and DUHAMEL. In tracing the ascent of sap he used a deep-colored infusion of grape skins to locate the translocating tissues, and also used ringing technique for studies of sap descent in the phloem. He was always critical in his judgments, and had a fine sense of disagreement with previous authors, and with the current beliefs of his day. He says: "In the authors I have looked into, I have seen many contradictory experiments related, and many conclusions drawn from a small number of facts, and I have found much that does not well agree with the things that have come under my own observation." Speaking of leaves he says: "This organ has much engaged the attention of naturalists, particularly Mr. BONNET: but their experiments have chiefly been made on leaves severed from the trees; and, therefore, whatever conclusions have been drawn, stand on very questionable ground." His papers are full of such critical statements.

In a paper on "*Experiments and Observations on the Motion of the Sap in Trees*" read in 1804, he mentions the eleventh plate in HALES' *Vegetable Staticks* disparagingly: "The experiments and still more, the Plates of HALES, have induced naturalists to draw conclusions in direct opposition to the preceding. But the Plates of that great naturalist are not always taken correctly from nature; and Plates, under any circumstances, however fair and candid the intentions of an author may be will too often be found somewhat better calculated to support his own hypotheses, than to elucidate the facts he intends to state." KNIGHT praises DUHAMEL highly for his accuracy, overlooking the fact, apparently, that DUHAMEL copied HALES' eleventh plate for his own work published

in 1758, and accepted it without question. Later KNIGHT was compelled to admit his own errors in connection with gravitational studies of sap movement in reversed stems.

The great paper on the influence of gravitation and centrifugal force on the responses of seedlings was read before the Royal Society in 1806. It bore the title: *"On the Direction of the Radicle and Germen during the Vegetation of Seeds."* The paper was not illustrated, but it is a classic of simplicity and clarity. The figure of his wheels presented in this paper is taken from Sir HUMPHREY DAVY'S *Agricultural Chemistry,* published in 1813. We quote the essential paragraphs as follows: "I conceived that if gravitation were the cause of the descent of the radicle, and of the ascent of the germen, it must act either by its immediate influence on the vegetable

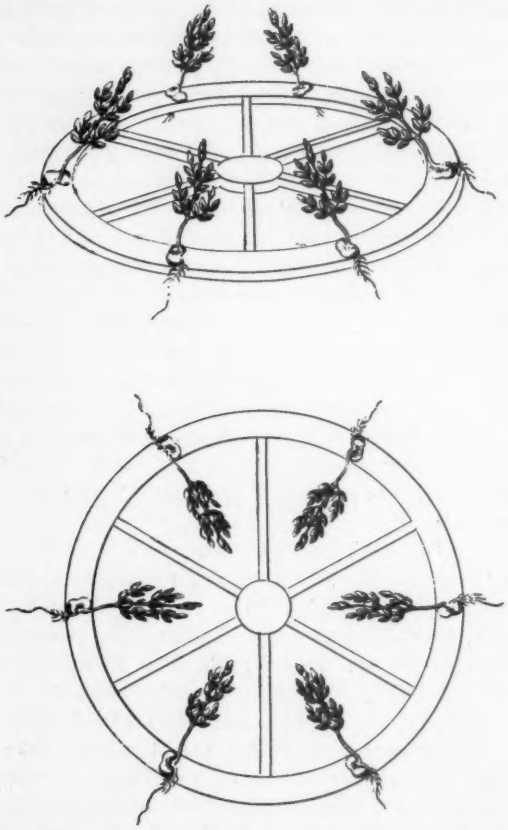


FIG. 1. Diagrammatic representation of KNIGHT'S rotating wheels, showing the direction of growth. From DAVY'S *Agricultural Chemistry.*

fibres and vessels during their formation, or on the motion and consequent distribution of the true sap afforded by the cotyledons: and as gravitation could produce these effects only whilst the seed remained at rest, and in the same position relative to the attraction of the earth, I imagined that its operation would become suspended by constant and rapid change of the position of the germinating seed, and that it might be counteracted by the agency of centrifugal force."

"Having a strong rill of water passing through my garden, I constructed a small wheel similar to those used for grinding corn, adapting a wheel of different construction, and formed of very slender pieces of wood, to the same axis. Round the circumference of the latter, which was eleven inches in diameter, numerous seeds of the garden bean, which had been soaked in water to produce their greatest degree of expansion were bound, at short distances from each other. The radicle of these seeds were made to point in every direction, some towards the center of the wheel, and others in the opposite direction; others as tangents to its curve, some pointing backwards, and others forwards, relative to its motion; and others pointing in opposite directions in lines parallel with the axis of the wheels. The whole was inclosed in a box, and secured by a lock, and a wire grate was placed to prevent the ingress of any body capable of impeding the motion of the wheels,. The water being then admitted, the wheels performed something more than 150 revolutions in a minute; and the position of the seeds relative to the earth was of course as often perfectly inverted, within the same period of time, by which I conceive that the influence of gravitation must have been wholly suspended."

"In a few days the seeds began to germinate, and as the truth of some of the opinions I had communicated to you, and of many others which I had long entertained, depended on the result of the experiment, I watched its progress with some anxiety, though with not much apprehension; and I had soon the pleasure to see that the radicles, in whatever direction they were protruded from the seed, turned their points outward from the circumference of the wheel, and in their subsequent growth receded nearly at right angles from its axis. The germens, on the contrary, took the opposite direction, and in a few days their points all met at the centre of the wheel. Three of these plants were suffered to remain on the wheel, and were secured to its spokes to prevent their being shaken off by its motion. The stems of these plants soon extended beyond the centre of the wheel; but the same cause, which first occasioned them to approach its axis, still operating, their points returned and met again at its centre."

"The motion of the wheel being in this experiment vertical, the radicle and germen of every seed occupied, during a minute portion of time in each revolution, precisely the same position they would have assumed had

the seed vegetated at rest; and as gravitation and centrifugal force also acted in lines parallel with the vertical motion and surface of the wheel, I conceived that some slight objections might be urged against the conclusions I felt inclined to draw. I therefore added to the machinery I have described another wheel which moved horizontally over the vertical wheels; and to this, by means of multiplying wheels of different powers, I was enabled to give many different degrees of velocity. Round the circumference of the horizontal wheel, whose diameter was also eleven inches, seeds of the bean were bound as in the experiment, which I have already described, and it was then made to perform 250 revolutions in a minute. By the rapid motion of the water wheel much water was thrown upwards on the horizontal wheel, part of which supplied the seeds upon it with moisture, and the remainder was dispersed, in a light and constant shower, over the seeds in the vertical wheel, and on others placed to vegetate at rest in different parts of the box."

"Every seed on the horizontal wheel, though moving with great rapidity necessarily remained in the same position relative to the attraction of the earth; and therefore the operation of gravitation could not be suspended, though it might be counteracted, in a very considerable degree, by centrifugal force; and the difference, I had anticipated, between the effects of rapid vertical and horizontal motion soon became sufficiently obvious, The radicles pointed downwards about ten degrees below, and the germens as many degrees above, the horizontal line of the wheel's motion; centrifugal force having made both to deviate 80 degrees from the perpendicular direction each would have taken, had it vegetated at rest. Gradually diminishing the rapidity of the motion of the horizontal wheel, the radicles descended more perpendicularly, and the germens grew more upright; and when it did not perform more than 80 revolutions in a minute, the radicle pointed about 45 degrees below, and the germen as much above, the horizontal line, the one always receding from, and the other approaching to, the axis of the wheel.

"I would not, however, be understood to assert that the velocity of 250, or of 80 horizontal revolutions in a minute will always give accurately the degrees of depression and elevation of the radicle and germen which I have mentioned; for the rapidity of the motion of my wheels was sometimes diminished by the collection of fibres of conferva against the wire grate; which obstructed in some degree the passage of the water: and the machinery, having been the workmanship of myself and my gardener, cannot be supposed to have moved with all the regularity it might have done, had it been made by a professional mechanic. But I conceive myself to have fully proved that the radicles of germinating seeds are made to descend, and their germens to ascend, by some external cause, and not by any power

inherent in vegetable life: and I see little reason to doubt that gravitation is the principal, if not the only agent employed, in this case, by nature."

In still others of his papers we find him solving problems that are refreshingly modern. One of them *"On the Inverted Action of the Alburnous Vessels of Trees"* contains an account of the profuse flowering even of very early varieties of potatoes, which ordinarily do not blossom, when he prevented the consumption of the carbohydrates by suppressing stolon formation. He accomplished the suppression in an ingenious fashion. Those who have given attention to the influence of clipping on forage survival and succeeding yields will appreciate this statement from a paper read in 1805: "I have constantly found, in my practice as a farmer, that the produce of my meadows has been immensely increased when the herbage of the preceding year had remained to perform its proper office till the end of the autumn, on ground which had been mowed early in the summer." And the relation of girdling to blossom-bud setting in apples receives the following comment: "It has been long known to gardeners that taking off a portion of bark round the branch of a fruit tree occasions the production of much blossom on every part of that branch in the succeeding season." If he had mentioned the high-carbohydrate content of these tissues he would truly have been a hundred years ahead of his time.

His breadth of interest is indicated by the fact that he wrote not only about many kinds of fruits and vegetables, but about insects, such as aphis and bees; and various plant diseases, such as blights, cankers, etc. He even interested himself in the formation of ice in the bottoms of rivers.

Born at Wormsley Grange, near Hereford, on August 12, 1759, son of THOMAS KNIGHT and grandson of RICHARD KNIGHT who won a fortune as an iron master, THOMAS ANDREW was educated at Ludlow School, later at Chiswick, and finally at Oxford as a matriculant in Baliol College. He was 20 years old at the time he entered Oxford. He was possessed of an inherent interest in all kinds of plants and animals and was an eager sportsman and excellent shot. He utilized these inclinations, however, only as an opportunity for studying nature. The facts and incidents collected at this early period contributed a fund of information which formed the basis of many of his subsequent investigations; and his possession of land and funds gave him every opportunity to follow his natural inclinations. After leaving Oxford he lived at Elton where all of his early papers were written. The last of his letters to Sir JOSEPH BANKS from Elton was read on February 23, 1809. The next succeeding contribution, read on June 22, 1809 was sent from Downton Castle, where THOMAS ANDREW had an estate of ten thousand acres which was deeded to him by his older brother, RICHARD PAYNE KNIGHT, who is best known as a numismatist.

While still at Elton, KNIGHT helped to found the Royal Horticultural Society in 1804 as a charter member, and became its president in 1811; this position he held for 27 years, until his death in 1838. He became a fellow of the Royal Society in 1805, was awarded the Copley medal in 1806, and was elected a Fellow of the Linnean Society in 1807. He was also awarded the first Knightian Medal of the Royal Horticultural Society, a medal that bears his portrait and which was founded and named in his honor. In addition to those mentioned above he received nine other medals and was a member of 20 Societies in various countries. All of these honors were richly deserved.

In 1791, at the age of 33, he married FRANCES FELTON whose gentleness of disposition and unceasing endeavor to promote his comfort and happiness was a major factor in his achievements. They had four children, three daughters and one son. The latter was accidently killed when near his 32nd year; this incident had a profound effect upon KNIGHT'S thought and work for many years. His wife and daughters survived him at his death.

Anyone who reads THOMAS ANDREW KNIGHT'S scientific contributions, his treatise *On the Manufacture of Cider and Perry* (1797), *A Treatise on the Culture of the Apple and Pear* (1797), *Pomona Herefordiensis* (1811), and his numerous practical papers on horticultural practices, must be impressed with his keen intellect, his unusual powers of observation, and his ability to express himself with force and clarity. His singular powers of memory, which he retained until his death, were of particular note. His skill in all kinds of horticultural operations, and his appreciation of the problems inherent in the production of fruits and vegetables, turned him to very practical services. One of his peculiarities was the readiness by which, with his own hands and the assistance of a common carpenter or blacksmith, he would construct all of the machinery required in his most elaborate experiments.

He placed England's horticulture on a solid foundation, and its subsequent growth and development is an enduring monument to his genius. As the years pass away, his memory will be preserved in the hearts and minds of all who are able to appreciate the time in which he lived. He stands as one of the most important contributors to our knowledge of natural history during the early part of the 19th century.

THE UNIVERSITY OF CHICAGO
CHICAGO TEACHERS COLLEGE
CHICAGO, ILLINOIS

ANTAGONISM OF CERTAIN ELEMENTS ESSENTIAL TO PLANTS TOWARD CHEMICALLY RELATED TOXIC ELEMENTS

ANNIE M. HURD-KARRER

(WITH FIVE FIGURES)

The hypothesis developed to explain the quantitative aspects of selenate-sulphate antagonism was based on the assumption of unselective absorption and utilization of the two ions by virtue of their chemical similarity (**18, 19**). The idea subsequently received support from plant analyses showing that the amount of selenium taken into the tissues of certain plants parallels that of sulphur, as though selenium absorption were determined by the sulphur requirement of the species (**20**). Reasoning by analogy to other pairs of chemically related elements, one of which is toxic and the other essential in nutrition, has led to the discovery that arsenate toxicity is a function of phosphate availability, rubidium toxicity of potassium availability, and strontium toxicity of calcium availability. In view of the small number of pairs that can be selected on such a basis, positive results with all three are highly suggestive of the validity of the basic assumption. The present paper presents the experimental evidence on which the previous brief reports of these relationships were based (**22, 23**).

Experimentation

INHIBITION OF ARSENIC TOXICITY BY PHOSPHORUS

Because their positions in the periodic table of elements are analogous to those of sulphur and selenium in an adjoining group, phosphorus, an essential element capable of being absorbed in relatively large amounts, and arsenic, a highly toxic element, chemically much like phosphorus and adjacent to it in division B of group V of the periodic table (**16**, p. 203), were chosen for experimentation.

The most obvious effect of arsenic on wheat was killing of the roots with withering and stunting of the tops, apparently as a result of root injury (fig. 1, D). The severely damaged leaves were narrow and stiff, and sometimes pale green in color.

The effect of phosphates on these symptoms was studied first in water cultures and then in several kinds of soil.

WATER CULTURES.—Seedlings of Hard Federation wheat were grown for several weeks in low-, medium-, and high-phosphate solutions containing disodium arsenate ($Na_2HAsO_4 \cdot 7\,H_2O$). The three nutrient solutions, with 10, 60 and 120 parts per million of phosphorus, contained, in 19 liters, 31, 184, and 367 cc. M/10 $CaH_4(PO_4)_2 \cdot H_2O$, respectively; plus 95 cc. M/5 $MgSO_4$,

FIG. 1. Wheat plants injured by rubidium (B), strontium (C), and arsenic (D), in comparison with control plant (A).

75 cc. M/1 NH_4NO_3, 60 cc. M/2 KCl, and 100 cc. M/60 ferric citrate. To equalize the differences in calcium, the first two of these solutions had, in addition, 67 and 37 cc. M/2 $CaCl_2$, respectively. They thus differed with respect to chlorine as well as phosphate; but the former did not affect the results, for it was highest in the low-phosphate solution, where it was shown by control cultures to be non-toxic.

The pH values of the low- and high-phosphate solutions were initially 6.6 and 5.7, produced by the addition of one and two drops, respectively, of sodium hydroxide to a 600-cc. culture.[1] After three weeks' growth of the seedlings in the solutions containing arsenic, the corresponding values were 5.7 and 5.2. These values seemed sufficiently similar to preclude any possibility that the differences in arsenic injury were due to acidity differences. Moreover, the same results were obtained when the initial pH value of the high-phosphate solution was brought to 6.5.

Each culture consisted of three seedlings in a flask containing 600 cc. of nutrient solution. Control cultures without arsenic were a part of each experiment, and showed that damage to the plants in the low-phosphate solutions containing arsenic was actually due to the arsenic in each case and not to phosphate deficiency.

That the degree of arsenic toxicity depended on the phosphate concentration in the nutrient solution is illustrated by the experiment of figure 2. On comparing the effects of a given absolute amount of arsenic at the three phosphate levels, it can be seen, for example, that the plants were almost killed by 30 p.p.m. of arsenic where there were only 10 p.p.m. of phosphorus available, slightly stunted with 60 p.p.m. of phosphorus, and uninjured with 120 p.p.m. The minimum arsenic concentrations causing extreme stunting were 10, 60, and 120 p.p.m. with phosphorus concentrations of 10, 60 and 120 p.p.m. respectively. This high degree of toxicity thus resulted from a 1:1 arsenic/phosphorus ratio irrespective of the absolute concentrations involved. In general, the arsenic was definitely toxic in the presence of less than four times as much phosphorus, but non-toxic where there was more than four times as much.

Injury was somewhat greater at a given ratio in the high-phosphate than in the low-phosphate series. That is, injury at a given ratio increased slightly with increasing concentration of arsenic.

Other experiments were carried out with both wheat and barley in solutions containing various concentrations of phosphate obtained by mixing,

[1] To guard against possible complicating effects of the difference in sodium necessitated by the pH adjustment, the low-phosphate cultures were duplicated in one experiment with the larger quantity of sodium hydroxide (2 drops) added to each. Arsenic toxicity proved to be of the same degree as that in the corresponding cultures with the smaller addition of sodium hydroxide, indicating that the effects attributed to phosphate differences were not due to the small difference in sodium concentration.

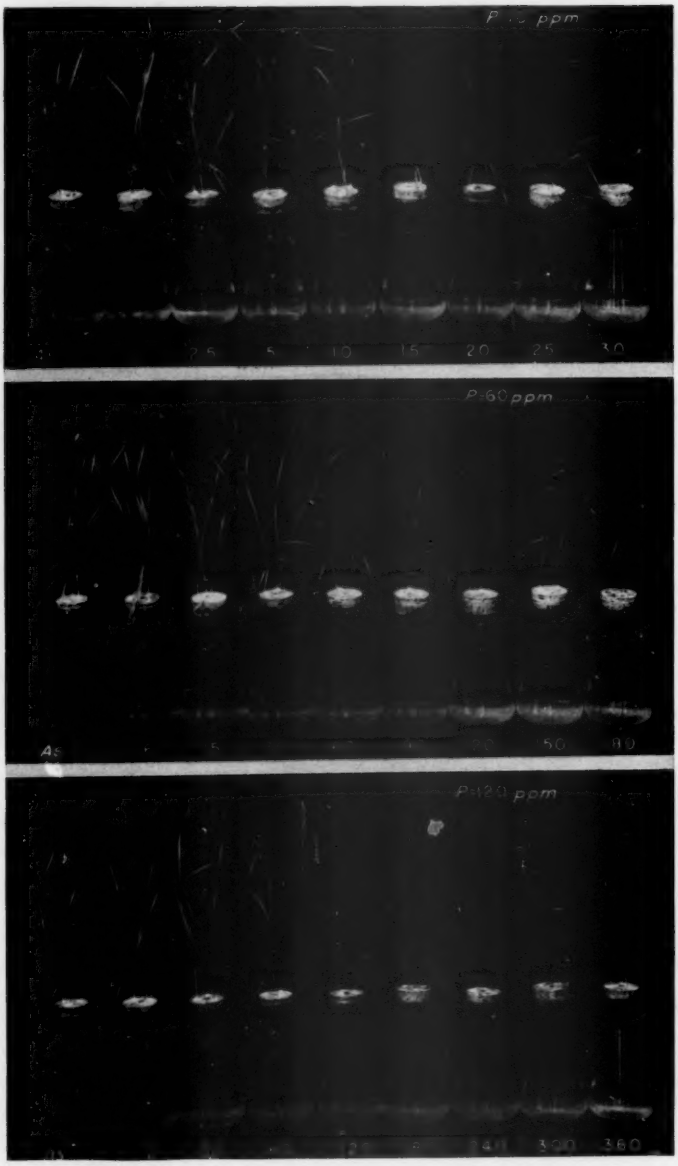


FIG. 2. Comparative toxicity of disodium arsenate to wheat in three different nutrient solutions, containing 10, 60 and 120 p.p.m. of phosphorus, respectively. The cultures in vertical alignment show the effect of a given As/P ratio. (Figures under flasks denote arsenic concentrations in p.p.m.)

TABLE I

THE TOXICITY OF SODIUM ARSENATE TO WHEAT AND BARLEY SEEDLINGS GROWN THREE WEEKS IN NUTRIENT SOLUTIONS OF DIFFERENT PHOSPHATE CONCENTRATIONS

EXPERIMENT	GREEN WEIGHT OF THREE PLANTS GROWN WITH PHOSPHORUS				
	10 p.p.m.	37.5 p.p.m.	65 p.p.m.*	92.5 p.p.m.	120 p.p.m.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1. Wheat					
Arsenic, 30 p.p.m. ...	0.50	.....	1.15	.....	2.16
Arsenic, 15 p.p.m. ...	0.75	.....	1.91	.....	2.51
Arsenic, none	2.67	.....	2.58	.....	2.38
2. Wheat					
Arsenic, 12.5 p.p.m.	0.70	.....	4.34	.....	4.78
Arsenic, none	3.75	.....	3.60	.....	5.12
3. Barley (fig. 3, A)					
Arsenic, 12.5 p.p.m.	0.38	.....	5.92	.....	6.21
Arsenic, none	6.08	.....	5.69	.....	6.34
4. Wheat (fig. 3, B)					
Arsenic, 30 p.p.m. ...	0.62	0.88	1.67	2.60	2.59
Arsenic, none	3.43	3.54	3.54	3.58	3.08

* This concentration was 60 p.p.m. instead of 65 p.p.m. in the first experiment.

in proper proportions, the low- and high-phosphate solutions. The data given in tables I and II, and the illustrations included in figure 3, all show the inverse relation between arsenic toxicity and phosphate concentration. Table II shows that the arsenic generally produced a greater reduction in growth at a given arsenic/phosphorus ratio (in comparison with corresponding controls without arsenic) when the solutions were renewed weekly than when they were unchanged.

TABLE II

THE TOXICITY OF 12 P.P.M. ARSENIC (AS SODIUM ARSENATE) TO ONE-MONTH-OLD WHEAT SEEDLINGS IN NUTRIENT SOLUTIONS OF DIFFERENT PHOSPHATE CONCENTRATIONS, WITH AND WITHOUT WEEKLY RENEWAL

PHOSPHATE AS P	APPROXIMATE As/P RATIO	GREEN WEIGHT OF THREE PLANTS			
		SOLUTIONS NOT RENEWED		SOLUTIONS RENEWED WEEKLY	
		WITH ARSENIC	WITHOUT ARSENIC (CONTROLS)	WITH ARSENIC	WITHOUT ARSENIC (CONTROLS)
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
65	1:5	2.43	2.44	2.40	3.21
51	1:4	2.34	2.23	2.51	2.89
37	1:3	1.82	2.68	2.53	3.05
24	1:2	1.42	2.17	1.50	2.40
10	1:1	0.78	2.48	0.79	2.65

It is concluded that under the conditions of these various experiments, a 1:1 arsenic/phosphorus ratio was almost lethal, a 1:2 ratio highly toxic, while a 1:5 ratio rendered the arsenic harmless. Because of the probability that some of the phosphate was precipitated as ferric phosphate, especially at the higher concentrations, these ratios are considered approximations only. However, they have been sufficiently constant for a given degree of injury to permit the conclusion that arsenic injury is determined by proportionate phosphate availability.

SOIL CULTURES.—Hard Federation wheat was grown in the greenhouse in 10-inch pots containing 6 kg. of soil on a layer of gravel. The arsenic as $Na_2HAsO_4 \cdot 7\,H_2O$ and the phosphate as $NaH_2PO_4 \cdot H_2O$ were added in solution, thoroughly stirred into the soil of each individual pot.

The first experiments were with the clay loam (Keyport) of the Arlington Experiment Farm, Rosslyn, Virginia. Improvement in the growth of the arsenic-injured plants was noted in pots with excess phosphate when the plants entered the jointing stage although it had not been apparent in younger stages. When the plants were cut after nine weeks' growth it was found that the toxicity of the arsenic was definitely reduced by the phosphate applications, as shown by somewhat higher dry weights and unretarded stages of development. The averaged plant weights showed that 125 and 250 p.p.m. of arsenic reduced yields to 54 and 20 per cent., respectively, of that of the controls. With phosphate applications corresponding to 125 p.p.m. of P, these yields were increased to 73 and 41 per cent. of those of similarly treated controls without arsenic.

The experiment was repeated with a sandy loam[2] in the expectation of more positive results in soil of lesser adsorptive capacity. The growth of the plants with several different concentrations of arsenate and phosphate is indicated in table III. In every case the addition of phosphate to the soil

TABLE III

THE EFFECT OF SODIUM PHOSPHATE ON THE TOXICITY OF SODIUM ARSENATE TO WHEAT (2 MONTHS OLD) IN A SANDY LOAM SOIL

ARSENIC	WEIGHTS OF 13 PLANTS WITH THE FOLLOWING CONCENTRATIONS OF PHOSPHATE (P.P.M. P) ADDED TO SOIL		
	0	100 p.p.m.	200 p.p.m.
p.p.m.	*gm.*	*gm.*	*gm.*
0	7.48	8.50	8.73
50	4.09	8.53	8.08
100	1.04*	3.14	6.30

* These plants were in the pre-heading stage. All others were flowering.

[2] Obtained from an artificial fill from the Potomac River on the Arlington Experiment Farm, Virginia.

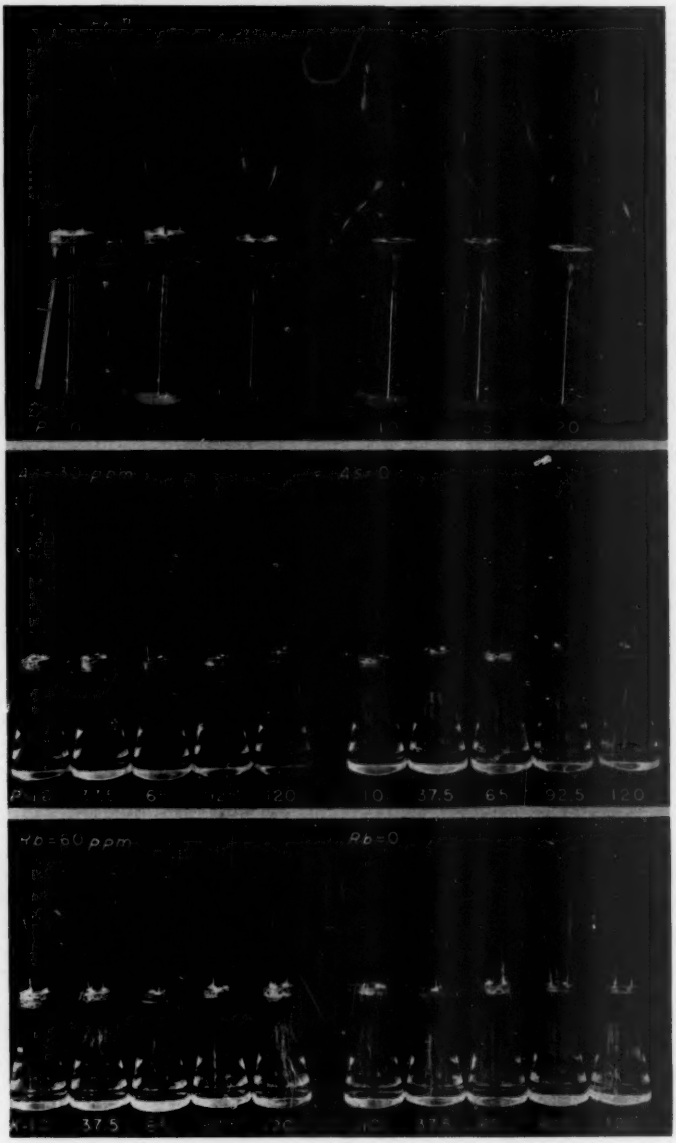


FIG. 3. Upper row: Effect of phosphate (10, 65, and 120 p.p.m. of P) on the toxicity of disodium arsenate (12.5 p.p.m. of As) to barley. Controls without arsenic to the right. Middle row: Effect of phosphate (10, 37.5, 65, 92.5, and 120 p.p.m. of P) on the toxicity of disodium arsenate (30 p.p.m. of As) to wheat. Controls without arsenic to the right. Lower row: Effect of potassium (10, 37.5, 65, 92.5, and 120 p.p.m. of K) on the toxicity of rubidium chloride (60 p.p.m. of Rb) to wheat. Controls without rubidium to the right.

greatly decreased the arsenic injury. The differences were apparent from the time the plants were but two weeks old.

In order to determine whether the progressive decrease in arsenic injury with increasing phosphate connoted a corresponding decrease in the arsenic taken into the leaves, the tops of these plants were analyzed[3] for arsenic. The series with 50 p.p.m. of arsenic gave the values 20, 18, and 15 p.p.m. for the phosphate levels of 0, 100, and 200 p.p.m. of P, respectively. With 100 p.p.m. of arsenic in the soil, the corresponding analyses were 15, 26, and 24 p.p.m. for the plants. The data thus show no significant effect of the phosphate on the amount of arsenic taken into the leaves. It is known that more arsenic accumulates in the roots than in the tops of plants (**32, 39**) so perhaps roots rather than tops should be analyzed in studying the arsenate-phosphate relation.

Another experiment comprised plants grown for five weeks in a still sandier soil, obtained from Gunston, Virginia, by Mr. J. D. REID of the Division of Soil Fertility. The arsenic proved to be so toxic in this soil that 50 p.p.m. practically killed the plants within a few weeks except where phosphate was added. One hundred p.p.m. of P definitely improved their condition, while 200 p.p.m. of P made them indistinguishable from the controls.

Thus the toxicity of sodium arsenate and the extent to which phosphate can inhibit it depend on the soil type. Apparently the explanation is to be found in the differing capacities of soils to adsorb arsenates and phosphates (**10, 13, 38**). GILE (**13**) has shown that the two are not adsorbed alike by a given soil, their relative adsorption depending on the nature of the soil colloids; for although phosphate seems to be adsorbed by both the aluminum and the iron of the colloids, arsenic is adsorbed chiefly by the iron.

In some South Carolina soils calcium arsenate injury is reported to be increased by phosphate, which acts apparently by combining with the iron in the soil, rendering it unavailable for insoluble combination with the arsenic (**10**). Other factors found to affect the toxicity of calcium arsenate in some of these soils are acidity, and reactive iron content. It is thus probable that the varying conditions for solubility, adsorption, and chemical combination of arsenic and phosphorus in soils render unpredictable the practical value of the arsenic-phosphorus antagonism. Most of the arsenic reaching the soil under field conditions is in the form of the comparatively insoluble calcium salt instead of the soluble sodium salt used in these experiments.

[3] The analyses were made by Mr. C. C. CASSIL with the Gutzeit method, through the courtesy of Dr. R. C. ROARK, Chief of the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine.

That the form in which the arsenic occurs is likely to be an important factor is suggested by the fact that sulphate more effectively reduces absorption of selenium from selenates than from selenites (**21**). By analogy, phosphates would be expected to have less effect on the toxicity of arsenites than on that of the arsenate used in these experiments.

It is obvious that the critical ratios determining growth in nutrient solutions do not apply directly to soils, as pointed out previously in connection with the selenium-sulphur antagonism. LIPMAN's critique of the "hypothesis of the lime-magnesia ratio" (**26**) is evidence of the difficulties and complications inherent in attempts to establish universally applicable ratios in soils.

INHIBITION OF RUBIDIUM TOXICITY BY POTASSIUM

Another pair of elements investigated comprised potassium, as the essential element, and rubidium, a moderately toxic element similar to potassium chemically and adjacent to it in division A of group I in the periodic table (**16**, p. 43).

The most characteristic symptom of rubidium injury was a peculiar thickening and stunting of the roots, especially of the crown roots (fig. 1, B). The leaves of severely injured plants were an abnormally dark green.

A preliminary experiment with a complete nutrient solution containing 94 p.p.m. of rubidium as rubidium chloride showed that the addition of an excess of potassium chloride to the cultures definitely reduced the toxicity of the rubidium. The green weights, in grams, of the three plants of duplicate cultures after three weeks were as follows:

	With 60 p.p.m. of K	*With 190 p.p.m. of K*
With 94 p.p.m. of rubidium	1.52	2.22
	1.57	2.16
Controls without rubidium	2.56	2.90
	2.71	2.45

In the second experiment the plants were grown with 100 p.p.m. of rubidium as rubidium chloride in solutions containing 10, 60, and 120 p.p.m., respectively, of potassium as KCl.[4] Rubidium injury was pronounced (especially that of the roots) with 10 p.p.m. of K, less with 60 p.p.m., and absent with 120 p.p.m. of K (fig. 4). The condition of the control plants showed that the injury ascribed to rubidium in the low-potassium cultures was not due to potassium deficiency.

[4] All ions except potassium and chlorine were equal in the three solutions. They each contained, in 19 liters, 184 cc. M/10 $CaH_4(PO_4)_2$, 120 cc. M/1 NH_4NO_3, 95 cc. M/5 $MgSO_4$, 37 cc. M/2 $CaCl_2$, and 100 cc. M/60 iron citrate,—with 10, 59, and 117 cc. M/2 KCl for the low-, medium-, and high-potassium levels, respectively. The initial pH values of the three solutions were all between 3.9 and 4.1.

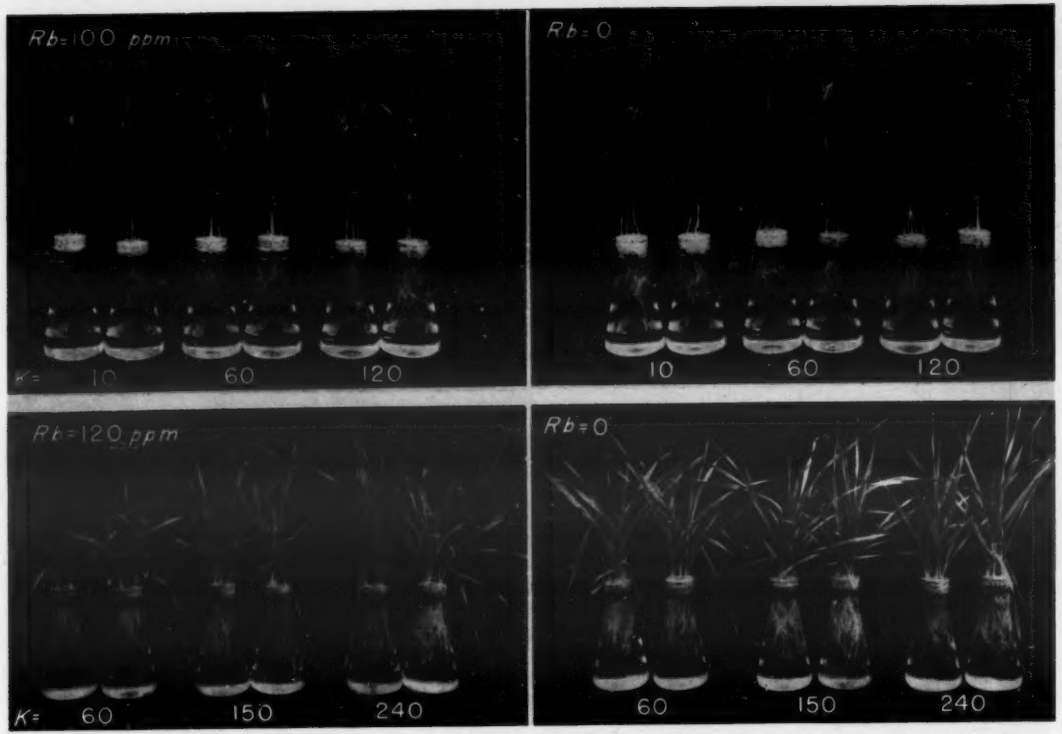


FIG. 4. Upper row: Effect of potassium (10, 60 and 120 p.p.m.) on the toxicity of rubidium chloride (100 p.p.m. of Rb) to wheat (duplicate cultures). Controls without rubidium to the right. Lower row: Effect of potassium (60, 150 and 240 p.p.m.) on the toxicity of rubidium chloride (120 p.p.m. of Rb) to barley (duplicate cultures). Controls without rubidium to the right.

Another experiment was set up with 60 p.p.m. of rubidium as rubidium chloride at five different potassium levels, obtained by mixing a low-potassium and a high-potassium solution in proper proportions.[5] Comparative degrees of rubidium injury to the plants are shown in figure 3 (lower row). The controls show that slight stunting of the tops resulted from potassium deficiency at the two lowest concentrations, 10 and 37.5 p.p.m., but the toxic effects of the rubidium in these solutions, especially those shown by the roots, were not obscured thereby. The rubidium injury was progressively reduced as the potassium in the solution increased, until with 92.5 p.p.m. the plants were as good as the corresponding controls without rubidium.

The experiment was repeated with 60 p.p.m. of rubidium in duplicate series of cultures, for one of which the solution was renewed every week, while for the other it was left unchanged for the duration of the experiment. The pH values were adjusted to 6.5, which was soon reduced to 4.3 where growth was vigorous and to 5.0 where it was not. The plants of both sets were cut after 6 weeks, at which time they were alike in showing rubidium injury in the low potassium solution and none where there was as much as 65 p.p.m. of potassium. An intermediate potassium concentration (37.5 p.p.m.) almost but not entirely inhibited the injury.

A final experiment was carried out with barley. Three potassium levels, 60, 150 and 240 p.p.m. as KCl were used, the lowest being made high enough to avoid symptoms of potassium deficiency.[6] The differences in chlorine were equalized with calcium chloride in duplicate series which provided incidentally an opportunity to determine whether calcium, known to have an ameliorating influence on a number of toxic conditions, might reduce rubidium toxicity and so refute the assumed specificity of the rubidium-potassium relation.

Since the potassium concentrations in these solutions were higher than in the preceding experiments, it was necessary to increase the rubidium concentration correspondingly in order to have a Rb/K ratio that would produce definite injury. So 120 p.p.m. of rubidium as RbCl was added to each culture except the controls.

[5] These two solutions contained the following salts, in 19 liters: 184 cc. M/10 $CaH_4(PO_4)_2$, 75 cc. M/1 NH_4NO_3, 95 cc. M/5 $MgSO_4$ and 100 cc. M/60 iron citrate, with 10 and 117 cc. M/2 KCl, producing 10 and 120 p.p.m. of potassium, respectively, in the low- and high-potassium solutions. The acidity was adjusted to pH 6.4 with sodium hydroxide.

[6] The low- and high-potassium solutions both contained, in 19 liters, 184 cc. M/10 $CaH_4(PO_4)_2$, 75 cc. M/1 NH_4NO_3, 95 cc. M/5 $MgSO_4$, and the iron citrate, the difference in potassium concentration being produced by 59 cc. and 234 cc. of M/2 KCl respectively. The intermediate potassium level was obtained by mixing the two solutions in equal proportions. The pH value of each was brought to 6.6 with a drop of a sodium hydroxide solution.

That this rubidium was toxic in the solution with 60 p.p.m. of potassium and non-toxic in that containing 150 p.p.m. is shown in figure 4. The flask to the left of each pair of cultures in these photographs is of the series in which chlorine differences were equalized by the addition of excess calcium chloride to the low-potassium solutions. Of interest is the fact that excess calcium (*cf.* the right-hand flask of the low-potassium pair) did not reduce rubidium toxicity.

Inhibition of strontium toxicity by calcium

Finally, the possibility of inhibiting the toxicity of strontium by calcium, an essential element of similar chemical properties (**16**, p. 56), was investigated. The two are adjacent in division A of group II of the periodic table.

Unlike the case with rubidium, strontium injury to the tops of the plants was always more pronounced than that to the roots. A curious effect frequently obtained was an increase in the number of tillers. With sufficiently high concentrations the plants were thick, short bunches of sometimes as many as twelve tillers (fig. 1, C) instead of the three to five normal ones of the control plants (fig. 1, A). Leaf symptoms, not always present on the young plants, were a mottled yellowish and grayish firing, first appearing at the tips.

Preliminary experiments showed strontium to be so slightly toxic that in order to obtain symptoms of injury in solutions containing enough calcium to prevent deficiency symptoms on the control plants, it was necessary to use very high concentrations. About 1000 p.p.m. of strontium as strontium chloride ($SrCl_2 \cdot 6\,H_2O$) were required to kill the plants in a solution containing 45 p.p.m. of calcium as calcium nitrate.

The comparative growth of some plants grown with 500 and 1000 p.p.m. of strontium in solutions containing 45, 300, and 500 p.p.m. of calcium as $Ca(NO_3)_2$, all at pH 5.0 and with the nitrogen differences equalized by NH_4NO_3, is shown in table IV.

TABLE IV

THE TOXICITY OF STRONTIUM CHLORIDE TO WHEAT IN RELATION TO THE CONCENTRATION OF CALCIUM IN THE NUTRIENT SOLUTION

Strontium	Weights of three plants with the following concentrations of calcium		
	45 p.p.m.	300 p.p.m.	500 p.p.m.
p.p.m.	*gm.*	*gm.*	*gm.*
0	9.04	8.35	9.18
500	2.73	6.38	6.75
1000	0.86	4.36	7.39

The extreme stunting produced by the strontium in the solution containing but 45 p.p.m. of calcium was markedly reduced by the higher calcium levels, but not entirely prevented. The reduction in injury was apparent not only from the plant weights but also from the inhibition of the leaf-tip firing which characterized the plants in the low-calcium solutions. Control plants showed the symptoms to be due to the strontium chloride and not to calcium deficiency.

In order to check the possibility that the toxic symptoms attributed to strontium might be due in part to the chlorine in the molecule, a series of plants was grown with sufficient potassium chloride to supply 414 p.p.m. of Cl, slightly more than was supplied by the strontium chloride in the 500-p.p.m. strontium solutions. The results were completely negative, the potassium salt producing no injury to the plants at any calcium level, and so justifying the assumption that the toxic symptoms produced by the strontium chloride were due to the strontium and not to the chlorine.

For the next experiment two nutrient solutions were made with 50 and 500 p.p.m. of calcium, respectively, as calcium nitrate, the nitrogen being equalized with ammonium nitrate.[7] An intermediate calcium concentration (275 p.p.m.) was obtained by mixing the two solutions in equal proportions. The strontium, 500 p.p.m., was added as the chloride in one series of triplicated cultures and as the nitrate in another. To check on possible effects of the anions, two other series were set up without strontium but with equivalent amounts of the chloride (405 p.p.m. of Cl) and nitrate (708 p.p.m. of NO_3) of potassium.

Both the appearance of the plants (fig. 5) and their weights (table V) when cut after three weeks' growth in the solutions showed that 500 p.p.m. of strontium produced considerable stunting with 50 p.p.m. of calcium, very little with 275 p.p.m., and none with 500 p.p.m. The series with the chloride and nitrate of potassium instead of strontium showed that neither of the anions was involved, the plants being uninjured and like the controls at each calcium level. The control plants showed that there was no stunting or other abnormality from insufficient calcium in the low-calcium solutions, but that the high-calcium solution was somewhat toxic.

While these results appear to indicate that the toxicity of strontium is inhibited by an equivalent amount of calcium, the critical ratio has not been determined over a range of concentrations. At high concentrations, a rather heavy precipitate developed, rendering exact statement of ratios of doubtful value.

[7] Both contained, in 19 liters, 50 cc. M/5 K_2HPO_4, 50 cc. M/5 KH_2PO_4, 95 cc. M/5 $MgSO_4$, and 100 cc. M/60 iron citrate. The low-calcium solution contained 47.5 cc. M/2 $Ca(NO_3)_2$ and 214 cc. M/1 NH_4NO_3; the high-calcium solution contained 475 cc. of the former and none of the latter. The initial pH values were 6.55 and 6.45 for the low- and high-calcium solutions, respectively.

TABLE V

THE TOXICITY OF 500 P.P.M. OF STRONTIUM TO WHEAT IN RELATION TO THE CONCENTRATION OF CALCIUM IN THE NUTRIENT SOLUTION

SALT ADDED TO NUTRIENT SOLUTION	TRIPLICATE WEIGHTS, EACH OF THREE PLANTS, WITH THE FOLLOWING CONCENTRATIONS OF CALCIUM		
	50 P.P.M.	275 P.P.M.	500 P.P.M.
	gm.	*gm.*	*gm.*
None (controls)	4.43	4.91	3.04
	4.08	4.27	3.60
	3.96	4.07	3.51
Average	4.16	4.42	3.38
Strontium chloride	2.19	2.94	3.38
	2.16	3.24	3.86
	2.50	4.00	3.12
Average	2.28	3.39	3.45
Strontium nitrate	2.28	3.95	3.45
	1.90	3.94	3.54
	2.29	3.10	3.87
Average	2.16	3.66	3.62
Potassium chloride	3.82	3.93	3.98
	4.32	4.09	3.53
	4.50	4.63	3.23
Average	4.21	4.22	3.58
Potassium nitrate	4.80	4.14	2.66
	4.53	4.45	3.78
	4.64	4.40	3.59
Average	4.66	4.33	3.34

The toxicity of barium also is inhibited by calcium (**12, 28, 29, 30, 37**), which would seem consistent with the present thesis. However, calcium seems to be such a general antagonizer (**29**) as to render uncertain the significance of its effects on its relatives, magnesium, strontium, and barium.

MISCELLANEOUS EXPERIMENTS

The question then arose as to whether phosphorus, potassium, and especially calcium were specifically antagonistic for arsenic, rubidium, and strontium, respectively, as assumed by the hypothesis which led to their selection for study, or whether their inhibiting effects might be more general. Accordingly arsenic toxicity was tested in the graded potassium and calcium solutions, rubidium toxicity with reference to phosphate and calcium levels, and strontium with reference to potassium. Only negative results were obtained, which seemed to corroborate the assumption of specificity in the antagonistic effects obtained with the chemically related pairs. However, MCCOOL obtained positive effects of potassium on strontium toxicity (**29**).

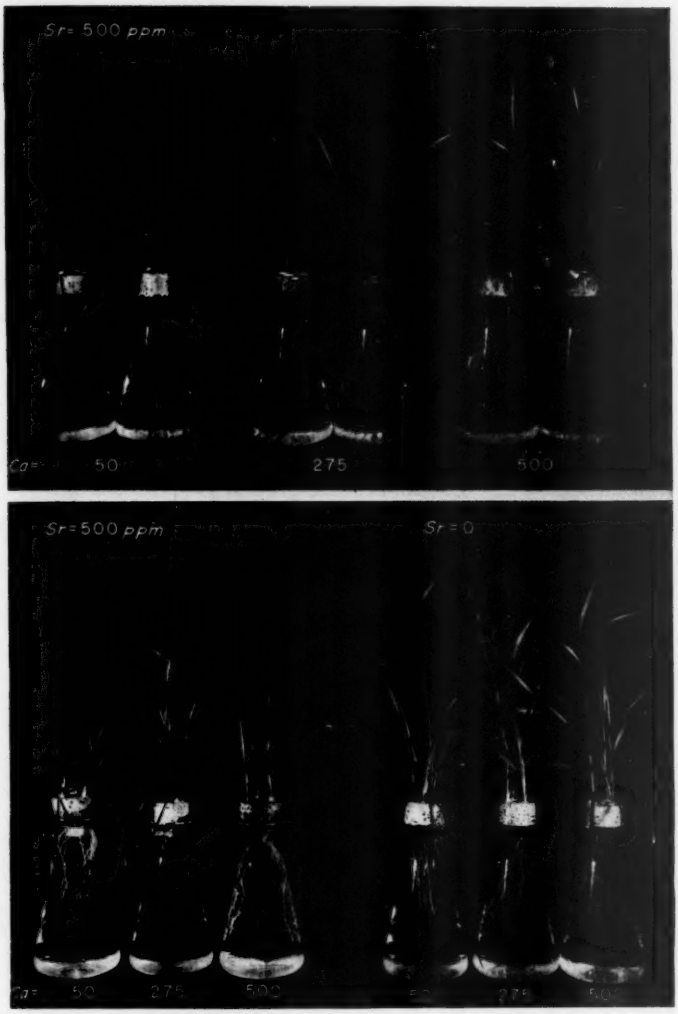


FIG. 5. Upper row: Effect of calcium (50, 275 and 500 p.p.m.) on the toxicity of strontium chloride (500 p.p.m. of Sr) to wheat in duplicate cultures. Lower row: Effect of calcium (50, 275 and 500 p.p.m.) on the toxicity of strontium nitrate (500 p.p.m. of Sr) to wheat. Controls without strontium to the right.

Discussion

The basic concept of what may be termed "mass antagonism" is simply that of a mass effect of an essential element on the proportionate intake, and utilization in organic syntheses, of a toxic element sufficiently similar chemically as to preclude any considerable selectivity. Large effects can be expected only with the major essential elements, which are absorbable in relatively large quantities without injury to the plant. The total intake of the two related elements, the one essential, the other toxic, is presumably determined by the gradient established by the plant's metabolism of the essential one; and the intake of the toxic element decreases, in consequence, with increasing availability of the non-toxic one. This relation has been shown by chemical analyses of plants to obtain between selenates and sulphates (**17, 19, 21**), and the assumption that a similar situation might obtain with respect to the salts of analogous pairs of elements initiated the present investigations.

The literature dealing with the mineral nutrition of plants describes many instances of the interdependence of absorption rates of the elements, and of various antagonistic effects, antagonism being defined as the hindrance of one salt on the absorption and toxicity of another. The researches of LOEB with animals and of LOEW and OSTERHOUT with plants are outstanding in this field. For summaries of some of the many contributions to the subject, reference is made to reviews already published (**11, 29, 35**).

Of these various instances of antagonism, an outstanding one is that of calcium and magnesium, which seems likely to be of the type described in the present paper. These two elements are adjacent to each other in division A of group II of the periodic table. LOEW (**28**) postulated harmful substitution of magnesium for calcium in a calcium-protein compound of the nucleus and chloroplast, with the protective action of excess calcium a simple mass effect. Similarly LOEB (**27**) assumed, in explanation of physiologically balanced solutions, that cations enter into combination with proteins and can be replaced by other kinds of cations from the external solution "in accordance with the law of the mass." KEARNEY and CAMERON (**25**) concluded that "the theory that ions furnished by the dissociation of electrolytes form intimate combinations with the proteids of protoplasm, and that their mutually antagonistic effect expresses itself in a replacement of one kind of ion by another as a result of change in the composition of the surrounding solution, would appear to afford the key to this problem," that is, the problem of "the effect of one kind of ion in counteracting the physiological action of another kind." MEYER's finding (**31**) that the relative rather than the absolute amounts of lime and magnesium available determine toxicity by virtue of absorption into the plant in the same ratio as that in which they occur in the soil is consistent with the idea.

The possibility of substitution of chemically related ions in plant metabolism has been rather thoroughly explored by early investigators, both with respect to selenium and sulphur, as referred to elsewhere (**18**), and the other three pairs of elements dealt with in the present paper.

JADIN and ASTRUC (**24**) assumed that arsenic forms compounds in plant cells analogous to those formed by phosphorus. They refer to earlier work by GAUTIER and BERTRAND as establishing the fact of such substitution of arsenic for phosphorus. BOUILHAC (**5**) and STOKLASA (**36**) concluded that arsenates may partially replace phosphates in nutrition because, in the absence of sufficient phosphate, growth is improved when some arsenate is present. The extreme toxicity of arsenic to protoplasm and the very slight capacity of plants to accumulate it in the leaves (**6**) seem to indicate that arsenic injury to the tops is mostly a secondary effect of injury to the roots (where analyses have shown most of the absorbed arsenic accumulates (**32, 39**) rather than the result of substitution of arsenic for phosphorus in synthetic processes in leaves.

With respect to rubidium and potassium there are reports by ALTEN and GOTTWICK (**1**), LOEW (**28**), BLANCK *et al.* (**4**), SCHARRER and SCHROPP (**33**), HELLER *et al.* (**15**), and ARNDT (**2**). The toxicity of rubidium was interpreted as evidence of its non-substitutability for potassium in nutrition. But incidentally the fact was noted by some (**2, 15**) that a high potassium content was associated with reduced rubidium intake and reduced injury. ARNDT (**2**) showed that rubidium toxicity is a function of the rubidium/potassium ratio, a 1:3 ratio rendering the rubidium non-toxic. These reports showed that rubidium does not replace potassium harmlessly. The observations of antagonism of rubidium injury by potassium can be interpreted as the mass effect of the latter on the chance of harmful substitution of rubidium in the synthesis of compounds normally formed with potassium.

A similar situation with respect to a strontium-calcium relationship was revealed by a search of the literature. The claim (**14**) that calcium could be replaced by strontium in the nutrition of the plant was questioned on the grounds of the toxicity of strontium; but incidentally the discovery was made that strontium injury could be reduced or prevented by increasing the available calcium above a certain level (**12, 28, 29, 30, 34, 37**), a fact in accord with the assumption of harmful substitution of strontium for calcium.

If harmful replacement of a nutritive element by a toxic element occurs, an excess of the former would be expected on theoretical grounds to reduce the symptoms produced by the toxic element by reducing the chances of the replacement occurring. That this consequence of substitution was not stated by the earlier investigators is probably due to the fact that harmless utilization was being sought, and the possibilities and implications of harmful replacement were not followed up. Their work demonstrated that the essential

elements are not functionally or physiologically replaceable, but they did not demonstrate chemical irreplaceability nor preclude the possibility that the plant injury observed was caused, in part at least, by such substitution.

There are several reports of decreased absorption of the essential element of these pairs in the presence of the more toxic one, as should be the case if the total were established by the plant's metabolism of the essential element, and if the proportionate intake of each depends on their proportionate availability. Thus MEYER (31) noted that fertilizing with magnesium reduced the calcium while increasing the magnesium content of the plant; HASELHOFF (14) noted that increasing the strontium in the nutrient solution decreased the calcium while increasing the strontium intake; and others (1, 15) found that increasing rubidium absorption was associated with decreasing potassium intake.

Analyses of plants of different species have shown direct correlations between absorption of the elements of some of these pairs. Selenium absorption has been found to parallel that of sulphur (20), potassium that of rubidium (9), and strontium that of calcium (9), suggesting that, by virtue of their chemical similarity, the intake of the toxic element of each pair is quantitatively determined by the particular species' requirement of the essential one. Significant also is the work of COLIN and LAVISON (8) showing some differences but also certain significant similarities in the absorption and translocation of strontium and calcium.

A survey of the literature on permeability indicates that the rates of penetration of chemically similar ions, such for instance as sodium and potassium, or rubidium and potassium, are not equal (7). The physical properties of ions and of the root membranes can reasonably be assumed to be factors in the physical aspects of penetration, resulting in inequality of absorption regardless of the degree of chemical relationship. The concept of antagonism proposed to explain the phenomena described in the present paper is based on the idea that the root membrane has no capacity for selectivity beyond that inherent in such physical properties. The dominant factor is seen to be the physiological demand of the plant for a particular element, transmitted to the root through what may be termed the metabolism gradient for that element. The chemical nature of the available elements would seem to determine their response to this gradient. Incapacity to discriminate between the closely related ones is assumed to result in indiscriminate utilization and consequent damage to the plant in proportion to the relative rather than absolute availability of the toxic element.

Summary

1. Arsenic injury is a function of the available phosphate concentration, with the protective arsenic/phosphorus ratio in nutrient solutions near 1:5.

2. Rubidium injury is a function of the available potassium concentration, with the protective rubidium/potassium ratio in nutrient solutions near 1:2.

3. Strontium injury is a function of the available calcium concentration, with the protective strontium/calcium ratio in nutrient solutions near 1:1.

4. As a working hypothesis to explain these effects, the assumption is made that they are the result of some degree of unselectivity in absorption and utilization of chemically related elements, with the result that when an organic molecule is synthesized, the chance of harmful substitution of the toxic element for the essential nutrient depends on the proportionate availability of the two. If the assumption proves to be correct, this particular type of antagonism may appropriately be designated "mass antagonism."

CEREAL CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY
U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

LITERATURE CITED

1. ALTEN, F., and GOTTWICK, R. Ein Beitrag zur Frage der Vertretbarkeit des Kaliums durch Rubidium und Caesium für die Pflanzenernährung. Ernähr. Pflanze **29**: 393–399. 1933.
2. ARNDT, J. Neue Untersuchungen über die Frage des Ersatzes des Kaliums in der Pflanzenzelle durch Rubidium sowie über das Chlorbedürfnis von Buchweizen und Gerste. Ernähr. Pflanze **18**: 177–180; 191–194. 1922.
3. BERNARDINI, L., and SINISCALCHI, A. Intorno all'influenza di vari rapporti fra calce e magnesia sulla sviluppo delle piante. Ann. R. Scuola Sup. Agr. Portici, 2 Ser., 8, 19 pp. 1908. Cited from abstract in Exp. Sta. Rec. **22**: 433. 1910.
4. BLANCK, E., GIESECKE, F., and HEUKESHOVEN, W. Vegetationsversuche mit Hafer zur Klärung der Frage nach der Vertretbarkeit des Kaliums durch Rubidium. Jour. Landw. **81**: 205–221. 1933.
5. BOUILHAC, R. Végétation de quelques algues d'eau douce. Ann. Agron. **24**: 561–602. 1898.
6. BRENCHLEY, W. E. Inorganic plant poisons and stimulants. 2nd ed. Cambridge University Press. 1927.
7. BROOKS, S. C. The rate of penetration of rubidium into living cells of *Valonia* and its relation to apparent ionic radii. Jour. Cell. and Comp. Physiol. **2**: 223–231. 1932.
8. COLIN, H., and LAVISON, J. R. Absorption comparée des sels de baryum, de strontium et de calcium par la plante vivante. Rev. Gén. Bot. **22**: 337–344. 1910.

9. Collander, R. Über die Kationenelektion der höheren Pflanzen. Ber. d. bot. Ges. **55**: 74–81. 1937.
10. Cooper, H. P., Paden, W. R., Hall, E. E., Albert, W. B., Rogers, W. B., and Riley, J. A. Effect of calcium arsenate on the productivity of certain soil types. South Carolina Agr. Exp. Sta. Ann. Rept. **44**: 28–36. 1931.
11. Eisenmenger, W. S. Toxicity, additive effects, and antagonism of salt solutions as indicated by growth of wheat roots. Bull. Torr. Bot. Club **55**: 261–304. 1928.
12. Faack, K. Beitrag zur Frage der Funktionen des Kalziums in der Pflanze. Mitt. Landw. Lehrkanz. k. k. Hochsch. Bodenkul. Wien **2**: 175–207. 1913.
13. Gile, P. L. The effect of different colloidal soil materials on the toxicity of calcium arsenate to millet. Jour. Agr. Res. **52**: 477–491. 1936.
14. Haselhoff, E. Versuche über den Ersatz des Kalkes durch Strontium bei der Pflanzenernährung. Landw. Jahrb. **22**: 851–861. 1893.
15. Heller, K., Peh, K., and Gürtler, F. Über die Aufnahme von Rubidium durch die Kartoffelpflanze. Zeitschr. Pflanzenernähr. Düng. Bodenk. A. Wiss. Teil **35**: 215–222. 1934.
16. Hopkins, B. Smith. Chemistry of the rarer elements. D. C. Heath and Co., New York. 1923.
17. Hurd-Karrer, A. M. Relation of sulphate to selenium absorption by plants. Amer. Jour. Bot. **25**: 666–675. 1938.
18. ———. Selenium injury to wheat plants and its inhibition by sulphur. Jour. Agr. Res. **49**: 343–357. 1934.
19. ———. Factors affecting the absorption of selenium from soils by plants. Jour. Agr. Res. **50**: 413–427. 1935.
20. ———. Selenium absorption by crop plants as related to their sulphur requirement. Jour. Agr. Res. **54**: 601–608. 1937.
21. ———. Comparative toxicity of selenates and selenites to wheat. Amer. Jour. Bot. **24**: 720–728. 1937.
22. ———. Inhibition of arsenic injury to plants by phosphorus. Jour. Wash. Acad. Sci. **26**: 180–181. 1936.
23. ———. Rubidium and strontium toxicity to plants inhibited by potassium and calcium respectively. Jour. Wash. Acad. Sci. **27**: 351–353. 1937.
24. Jadin, F., and Astruc, A. Quelques considérations sur la présence, la répartition, le rôle de l'arsenic et du manganèse dans le règne végétal. Revue Sci. **54**: 589–593. 1916.
25. Kearney, T. H., and Cameron, F. K. The effect upon seedling plants of certain components of alkali soils. U. S. Dept. Agr. Report **71**: 7–60. 1902.

26. LIPMAN, C. B. A critique of the hypothesis of the lime-magnesia ratio. Plant World **19**: 83–105; 119–135. 1916.
27. LOEB, J. On ion-proteid compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution. Amer. Jour. Physiol. **3**: 327–338. 1900.
28. LOEW, O. The physiological rôle of mineral nutrients in plants. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 45. 1903.
29. MCCOOL, M. M. The action of certain nutrient and non-nutrient bases on plant growth. I. The antitoxic action of certain nutrient and non-nutrient bases with respect to plants. Cornell Univ. Agr. Exp. Sta. Mem. 2. 121–170. 1913.
30. MCHARGUE, J. S. Effect of certain compounds of barium and strontium on the growth of plants. Jour. Agr. Res. **16**: 183–194. 1919.
31. MEYER, D. Untersuchungen über die Wirkung verschiedener Kalk- und Magnesiaformen. Landw. Jahrb. **33**: 371–404. 1904.
32. NOBBE, F., BAESSLER, P., and WILL, H. Untersuchungen über die Giftwirkung des Arsen, Blei und Zink im pflanzlichen Organismus. Landw. Vers.-Sta. **30**: 381–423. 1884.
33. SCHARRER, K., and SCHROPP, W. Sand- und Wasserkulturversuche mit Lithium und Rubidium unter besonderer Berücksichtigung einer etwaigen Ersetzbarkeit des Kaliums durch diese beiden Elemente. Ernähr. Pflanze **29**: 413–25. 1933.
34. ————, and ————. Über die Wirkung von Strontium und Barium-Ionen auf das Wachstum einiger Pflanzen. Bodenk. u. Pflanzenernähr. **3**: 369–385. 1937.
35. STILES, W. Permeability. New Phytol. **22**: 128–149. 1923.
36. STOKLASA, J. De la substitution de l'acide arsénique à l'acide phosphorique dans la nutrition de la plante. Ann. Agron. **23**: 471–477. 1897.
37. SUZUKI, U. Can strontium and barium replace calcium in phanerogams? Bull. Coll. Agr. Tokyo **4**: 69–79. 1900.
38. TIULIN, A. TH. Availability of soil phosphates for the plant from the viewpoint of colloid chemistry. Soil Sci. **42**: 291–299. 1936.
39. VANDECAVEYE, S. C., HORNER, G. M., and KEATON, C. M. Unproductiveness of certain orchard soils as related to lead arsenate spray accumulations. Soil Sci. **42**: 203–216. 1936. Also, ALBERT, W. B., and PADEN, W. R. Calcium arsenate and unproductiveness in certain soils. Science n.s. **73**: 622. 1931.

RÔLE OF NITROGEN IN FUNGOUS THERMOGENESIS[1]

JOHN O. GASKILL AND JOSEPH C. GILMAN

(WITH TWELVE FIGURES)

Introduction

Thermogenesis by fungi has been definitely proved by MIEHE (**10, 11**), JAMES (**7, 8**), JAMES, RETTGER and THOM (**9**), NORMAN (**12**), GILMAN and BARRON (**3**) and others. The relation of substrate-composition to the thermogenic activity of the fungi involved has, however, received but little attention. Although the data presented by HARRISON (**4**) and those by NORMAN (**13**) clearly indicated the dependence of this process on the carbohydrate content of the substrate; the relationship that the nitrogen content, both as to type and amount, had to this process had not been investigated.

Various investigators have recognized that nitrogen added to substrates high in cellulose, hemicellulose, and similar plant products, bears an important relation to decomposition of these materials by fungi. BARTHEL and BENGTSSON (**1**) found that the favorable influence of stable manure upon cellulose fermentation in the soil was exerted by the nitrogen added with the manure. The soil micro-flora in this case was not limited to fungi. WAKSMAN (**17**) also found that nitrogen additions greatly increased the cellulose-decomposing activity of the soil micro-flora, except under anaerobic conditions. WAKSMAN and DIEHM (**18**), working with pure cultures of various fungi growing in a sand substrate containing additions of hemicellulose compounds and nitrogen, found that the ratio of nitrogen assimilated to hemicellulose decomposed was 1:35.5 for mannan, 1:16.7 for xylan, and approximately 1:35 for galactan. Further, in an experiment with fungous cultures growing in a liquid medium composed of ground corncobs with additional nitrogen, these investigators found that the ratio of nitrogen assimilated to corncob-xylan decomposed was 1:34.5. NORMAN (**13**) grew a number of fungi on oat straw with added ammonium carbonate and found that the "nitrogen factor" ranged from 0.50 for a species of *Phoma* to 0.83 for *Aspergillus terreus* Thom. He found, further, that the "nitrogen equivalent" was 3.33 for *A. terreus*—about an average for the fungi studied. This figure corresponds to a ratio of 1 part nitrogen to 30 parts organic matter and is quite similar to that determined by WAKSMAN and DIEHM for hemicellulose.

Although considerable work has been done on the relation of nitrogen to fungous decomposition of cellulose and related materials, comparatively few

[1] Journal paper no. J–512 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 85.

investigations have been made with reference to its rôle in thermogenesis. JAMES, RETTGER and THOM (9) found that, in the process of microbial heating in unsterilized cracked corn, total nitrogen remained practically constant, but that ammonia nitrogen very markedly increased, indicating a considerable transformation of organic nitrogen to the ammonia form, which, however, was not removed as such from the flask. NORMAN (12) working on thermogenesis of several fungi in cultures of oat straw, added nitrogen to the substrate in the form of ammonium nitrate, but did not test the influence of different amounts of nitrogen.

The problem was investigated in two of its aspects: first, the influence of various kinds and amounts of nitrogen upon temperatures attained in insulated cultures; and second, the influence of these compounds upon the general activity of the fungi, as measured by the loss in dry weight effected by them in substrates of similar composition.

Material and methods

CULTURES AND SUBSTRATE EMPLOYED IN THE EXPERIMENTS

One culture each of *Aspergillus flavus* Link, *A. terreus* Thom, *Penicillium oxalicum* Currie and Thom, and *Rhizopus tritici* Saito was obtained from J. W. HARRISON of the Botany Department of Iowa State College in September, 1931. These fungi had been isolated from alfalfa hay in the summer of 1930, and were chosen because of their thermogenic capacity (4).

In order to study the effect of nitrogen upon the thermogenic activity of these organisms, a substrate low in nitrogen was required, yet one suitable in other respects for their rapid development, and sufficiently porous to permit aeration in thermos-flask cultures. Corncob meal was found to be satisfactory. Well-dried corncobs of the 1930 crop were obtained from the Agronomy Farm of the Iowa Agricultural Experiment Station and ground in a Wiley mill to pass a sieve with 2-mm. apertures. The meal was then carefully mixed and stored in earthenware jars in the laboratory.

Corncobs are composed primarily of cellulose, pentosans, and lignin. Representative samples of the meal, prepared as described above, were analyzed for moisture, total nitrogen and ash content, with the following results:

Moisture (varying during the year)	7.10–9.43 per cent. of total weight
Total nitrogen (Kjeldahl method)	0.40 per cent. of dry weight
Ash	1.24 per cent. of dry weight

Moisture content is expressed as per cent. of the total weight, but because of the variation in moisture during the year and since all figures relating to nitrogen in the following experiments are referred to moisture-free meal, the percentages of the other two components are based on dry weight. The

percentage of total nitrogen, as here determined, corresponds rather closely to that given by HENRY and MORRISON (5) as an average figure. They state that elemental nitrogen comprises approximately 16 per cent. of crude protein, and thus the figure which they give is equivalent to 0.36 per cent. total nitrogen (on the dry weight basis), as compared with 0.40 per cent. obtained from the meal used in these experiments.

For the experiments with thermogenesis, 70-gm. samples of corncob meal were placed in 500-ml. Erlenmeyer flasks, which were plugged with cotton and sterilized by autoclaving one hour at 15 pounds pressure on each of four consecutive days. The samples were then poured into one-pint thermos flasks which had been autoclaved one and one-fourth hours at 15 pounds. For experiments with loss in dry weight, 5-gm. samples were placed in 50-ml. Erlenmeyer flasks, which were plugged with cotton, and autoclaved 30 minutes at 15 pounds on each of four consecutive days.

To provide the desired moisture content and to add the proper amount of nutrients, solutions of the necessary ingredients were prepared, placed in containers of convenient size, and autoclaved 25 minutes at 15 pounds. These were later mixed into the samples of meal. In the thermogenesis experiments, however, this step was delayed, as described later in connection with the inoculation process. The moisture content was brought to approximately 70 per cent. in all cases, since this percentage of moisture had been found in preliminary trials to be satisfactory for both the thermogenesis and the loss-in-dry-weight experiments.

INOCULATION AND GROWTH OF THE CORNCOB-MEAL CULTURES

To prepare inoculum, cultures of the organisms were grown at laboratory temperature on potato-dextrose-agar slants for a sufficient length of time to permit abundant sporulation. In the thermogenesis experiments, inoculation was performed in the following manner: single tubes of inoculum were used for each sample of meal, spore suspensions were prepared in the nutrient solutions mentioned above and poured into the thermos flasks containing the sterile meal. The flasks were temporarily closed with rubber stoppers, which had been washed in 50-per cent. alcohol, and were thoroughly shaken to mix the spores and medium and to provide for better aeration. The flasks were allowed to remain in a horizontal position for approximately one-half hour, and were rotated from time to time to facilitate absorption of the solution. Each flask was then fitted with a cotton plug, a thermometer, and an aeration tube consisting of a section of capillary glass tubing of suitable length, with the lower tip completely recurved to prevent stoppage by particles of meal. Both the thermometer and the aeration tube were washed by immersion in 50-per cent. alcohol before insertion into the flask. The bulb of the thermometer and the curved end of the aeration tube were lowered to

a point near the bottom of the flask. Duplicate platings were made from each culture at the close of all experiments to demonstrate the presence or absence of contaminants.

Since each loss-in-dry-weight experiment required the inoculation of a relatively large number of samples, the needle method was employed in preference to the use of spore suspensions. After the nutrient solutions had been introduced into the samples of sterile cob meal, the flasks were shaken sufficiently to loosen the meal and to facilitate aeration; a mass of spores and hyphae of convenient size was then introduced into the center of the substrate by a single stab. All cultures for loss-in-dry-weight determinations were made in duplicate.

The term "check" was used in the results from all experiments to indicate comparable uninoculated samples. A single check was used in each thermogenesis test, except where otherwise indicated in the results; in the experiments on loss in dry weight all checks were in duplicate.

After inoculation, the thermos flasks were placed on a laboratory table and subjected to the influence of varying room temperatures; however, since all cultures of any given experiment were handled at the same time, the results obtained within each experiment are comparable. In addition to the aeration by diffusion through the cotton plugs, a slow current of air was drawn through the cultures for one-half hour each day by means of the vacuum pump. The rate of aeration was controlled by bubbling the air from each aeration tube through dilute sulphuric acid. The amount of air drawn through the cultures in this manner was approximately 1.8 liters per day. Temperatures were recorded from two to four times each day, depending upon the rapidity of the temperature change, and charts were prepared from these detailed data. The figures, however, indicate only significant points and general trends.

All cultures in the loss-in-dry-weight experiments, except in preliminary trials, were incubated at room temperature for a period of 28 days. Dry weights were determined by oven-drying at 100° C. for 48 hours, and the actual loss in dry weight was calculated by direct comparison with checks handled in a similar manner and oven-dried at the same time. Percentage loss in dry weight was computed on the basis of original dry weight of the meal in each culture.

Experimental results

Comparison of the influence of additions of a single nitrogenous compound and a full-nutrient solution upon thermogenesis

In order to study the comparative effects of additions of a full-nutrient combination and of a single nitrogenous compound, alone, two nutrient solutions were prepared as follows:

Solution (1)

$Ca(NO_3)_2 \cdot 4H_2O$	9.0 gm.
H_2O (distilled) to make	1000.0 ml.

Solution (2)

$Ca(NO_3)_2$	9.0 gm.
KH_2PO_4	3.0 gm.
KCl	1.5 gm.
$MgSO_4 \cdot 7H_2O$	1.5 gm.
$FeSO_4$	0.03 gm.
Dextrose ($C_6H_{12}O_6$)	45.0 gm.
H_2O (distilled) to make	1000.0 ml.

These solutions were sterilized by autoclaving in the usual manner. Two samples of meal received, respectively, 50-ml. lots of solution (1) and (2), together with sufficient sterile water to give the moisture content of 70 per cent. and were inoculated with *Aspergillus flavus*. Calcium nitrate was the only source of nitrogen in each solution, and the rate of addition was 0.08 gm. elemental nitrogen per 100 gm. dry meal.

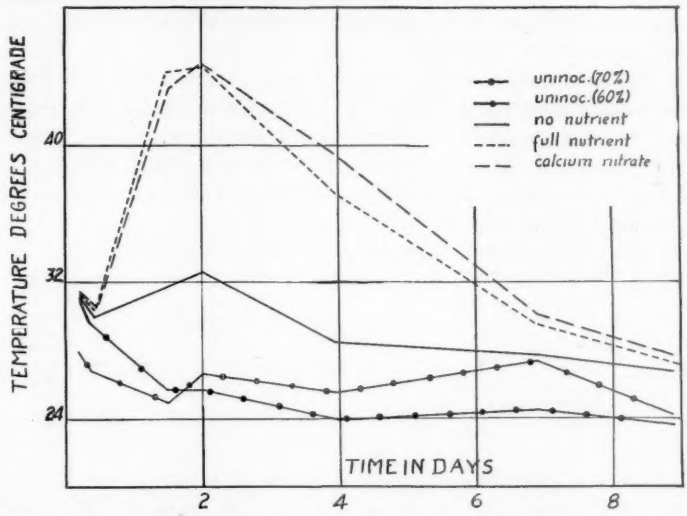


FIG. 1. Influence of different nutrient additions on temperatures in corncob meal cultures of *Aspergillus flavus*.

The results are presented in figure 1. The check, with moisture content equivalent to that in the two cultures, showed considerable fluctuation, which was found later to be caused by a faulty thermos flask. Another part of this experiment, dealing with the influence of moisture content upon thermogenesis, was being conducted at the same time; temperatures recorded for the check with 60 per cent. moisture are shown merely as indicative of the trend which temperatures in the 70 per cent. check normally would have taken. The results obtained from the inoculated cultures indicated that,

in this experiment, nitrogen, or at most calcium nitrate, alone, was the limiting factor in thermogenesis of *A. flavus*, since the addition of other nutrients resulted in but little difference in the temperatures reached. The culture containing the additional calcium nitrate without other nutrient additions reached its peak temperature of 44.75° C. at the end of the second day—18.25° above that of the check and 12.25° higher than the peak reached by the culture without added nutrient. The fact that nitrogen, and not calcium alone, was responsible for this pronounced stimulation of thermogenesis was demonstrated in subsequent experiments.

Influence of varying additions of nitrogen

When it was shown that the addition of a single nitrogenous compound to the meal greatly increased thermogenesis of *A. flavus*, experiments were made to test the effects of adding nitrogen in varying amounts. Three general types of nitrogenous compounds were separately employed, namely, organic-, ammonium- and nitrate-forms.

Thermogenesis.—Since the number of thermos flasks available was limited, only one organism, *A. flavus*, was used for experiments dealing with the influence of varying additions of nitrogen upon thermogenesis. Three experiments were conducted, in each of which the three compounds—asparagine, mono-ammonium phosphate ($NH_4H_2PO_4$), and calcium nitrate ($Ca(NO_3)_2 \cdot 4\,H_2O$)—were tested.

The results are presented in figure 2. Additions of asparagine ranged

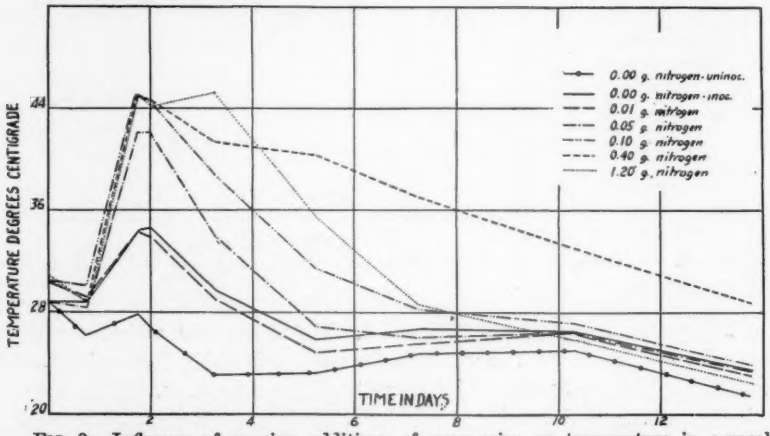


Fig. 2. Influence of varying additions of asparagine on temperature in corncob meal cultures of *Aspergillus flavus*.

from none to 1.20 gm. nitrogen per 100 gm. of dry meal, the upper limit being imposed by its low solubility. The pronounced increase in thermogenesis

with the additions of a single nitrogenous compound is again shown. All cultures containing asparagine additions of 0.10 gm. or more per 100 gm. of dry meal reached temperatures of 44.75° C. or higher—more than 10° above the peak reached by the culture without added asparagine and 17.5° or more above the check. Additions of asparagine in excess of 0.10 gm. nitrogen did not materially alter the rapidity of the response or the temperature peak, although a considerable prolongation of the period of heat production occurred in the culture containing the 0.40-gm. addition. The second peak and subsequent rapid fall in temperature of the culture receiving 1.20 gm. nitrogen may have been caused by a bacterial contaminant which was found at the close of the experiment. At this time the originally introduced organism seemed to be very largely destroyed, as evidenced by platings. Whether or not the contaminant could have had any appreciable influence upon the first peak in temperature is a matter of conjecture, but beginning with only a small amount of inoculum, it seems doubtful that it should have had any appreciable effect before the end of the second day.

The experiment with varying additions of mono-ammonium phosphate was similar to that in which asparagine was employed, except that additions of the nutrient were made over a much wider range—from none to 3.20 gm. nitrogen per 100 gm. of dry meal. The results are shown in figure 3. The differences observed between cultures receiving from 0.10 to 1.60 gm. nitrogen may be attributed to experimental error, such as that resulting from

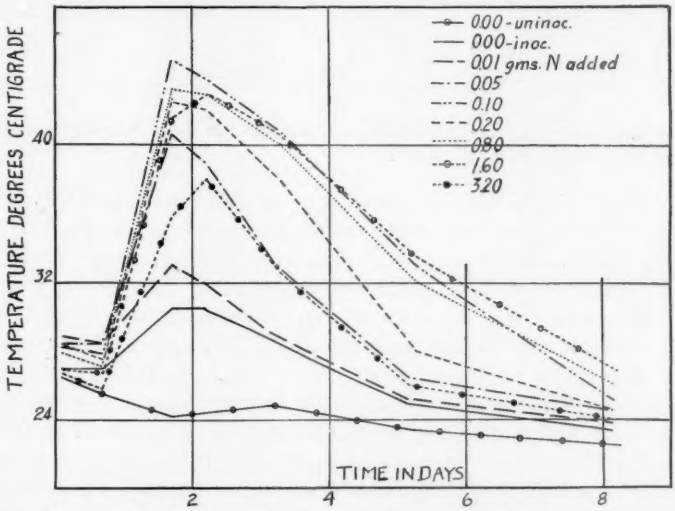


FIG. 3. Influence of varying additions of mono-ammonium phosphate on temperatures in corncob meal cultures of *Aspergillus flavus*.

slight differences in thermos flasks, in distribution of inoculum, and in conditions for aeration within the cultures. It is obvious, however, that 3.20 gm. nitrogen is well beyond the optimum. In this experiment, an addition of nitrogen as low as 0.01 gm. apparently resulted in a slight increase in thermogenesis, contrary to the experiment with asparagine.

In the third experiment of this series, varying additions of calcium nitrate were employed throughout the same range as with ammonium phosphate; and the results obtained (fig. 4) were quite similar. In these two experi-

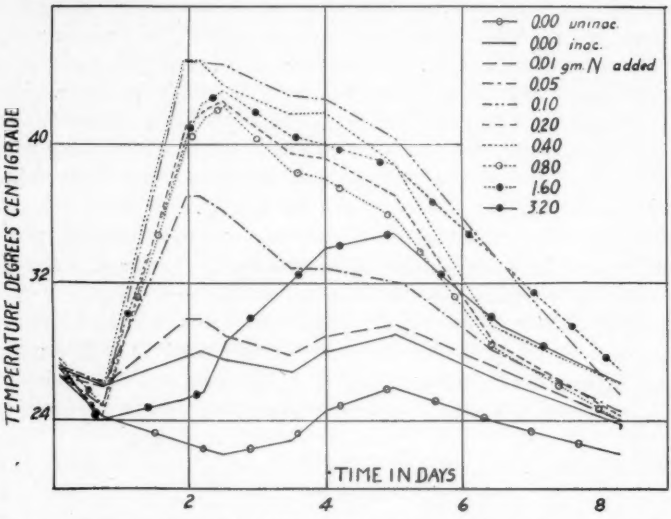


FIG. 4. Influence of varying additions of calcium nitrate on temperatures in corncob meal cultures of *Aspergillus flavus*.

ments, in which inorganic nitrogenous compounds were used, additions ranging from 0.10 to 1.60 gm. nitrogen per 100 gm. of meal produced only slightly differing results. Additions of 3.20 gm. and of 0.05 gm. nitrogen and below were decidedly less effective, although the amount of heating was markedly higher than where no nitrogen was added. With asparagine as with the inorganic compounds, additions ranging from 0.10 to 1.20 gm. nitrogen resulted in but slightly differing maximum temperatures. A decided prolongation of the period of heating occurred, however, in the one experiment where 0.40 gm. of nitrogen was added, as compared with 0.10 gm. The fact that the culture containing asparagine in the amount of 0.01 gm. nitrogen reached a maximum temperature slightly lower than the culture with no added nitrogen is probably not significant.

LOSS IN DRY WEIGHT.—In connection with those experiments in which varying nitrogen additions were tested for influence upon thermogenesis by

A. flavus, another series of experiments was conducted dealing with the effects upon fungous activity of varying additions of the same nitrogenous compounds, as shown by loss in dry weight. In these experiments relatively large numbers of cultures could be handled at one time, and thus all four of the fungi were employed in each test.

Before beginning with this series, however, a single experiment was carried out with *A. flavus* to determine the length of the growth period required for satisfactory comparisons in dry-weight loss. Four series of cultures—16 in each series—to which ammonium carbonate had been added at the respective estimated rates of 0.17, 0.22, 0.28, and 0.35 gm. of nitrogen per 100 gm. dry meal, and one series receiving no added nitrogen, were grown in comparison with uninoculated checks. Because of loss of ammonium carbonate in autoclaving, the amounts of nitrogen added to the cultures in the different series were estimated from total-nitrogen analyses made on ammonium-carbonate solutions prepared and autoclaved in exactly the same manner as those added to the cob-meal samples. At weekly intervals for a period of eight weeks, moisture-content and dry-weight determinations were made for one pair of cultures from each of the five series. At the end of eight weeks the rate of dry-weight loss in each series had apparently become almost nil and no loss whatever had occurred in the checks. By the end of four weeks dry-weight loss in every case was considerably greater than half the total loss reached in eight weeks and the relative position of each series, with respect to dry-weight loss, was very nearly the same throughout the period from the end of the third to the end of the eighth week. It seemed safe to conclude that a four-weeks' growth period should be sufficient for similar comparative tests, and consequently this period of growth was used in all subsequent experiments dealing with loss in dry weight.[2]

The influence of varying additions of asparagine upon dry-weight-loss effected by the four fungi—*A. flavus, A. terreus, Penicillium oxalicum,* and *Rhizopus tritici*—is shown in figure 5. In this experiment, as with that pertaining to the effects of asparagine upon thermogenesis, the low solubility of asparagine prevented any addition of this nutrient in a quantity greater than 1.2 gm. of nitrogen per 100 gm. dry meal. In every case progressive increases in dry-weight loss with larger additions of asparagine, up to 0.8 or 1.2 gm. nitrogen per 100 gm. of meal, occurred.

Figure 6 shows the results obtained in an experiment to determine loss in dry weight of cultures supplied with varying additions of mono-ammonium phosphate. The test was similar to that dealing with the effect of aspara-

[2] The standard error of a mean of two determinations, ± 0.3002, calculated on the basis of ten cultures of *A. flavus* grown under the conditions of these experiments showed that the inoculating technique employed was sufficiently accurate, considering the magnitude of dry-weight losses. It is also evident that, under similar conditions, only relatively small differences between pairs of cultures would be required to be significant.

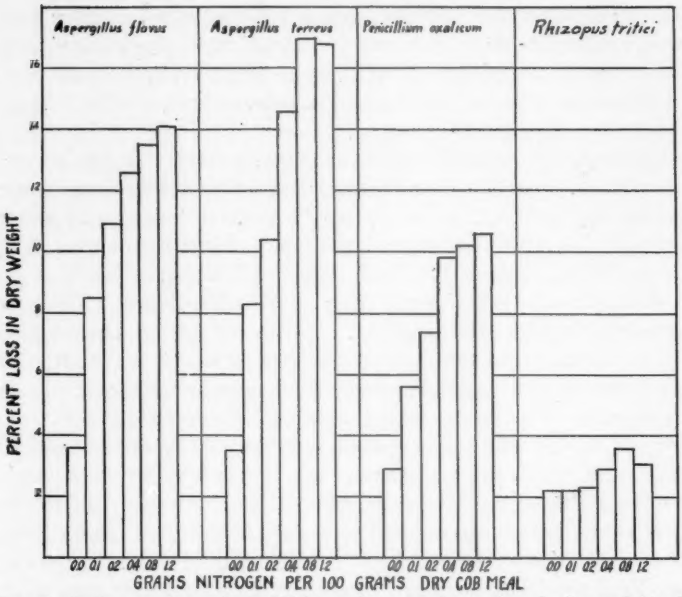


FIG. 5. Influence of varying additions of asparagine on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

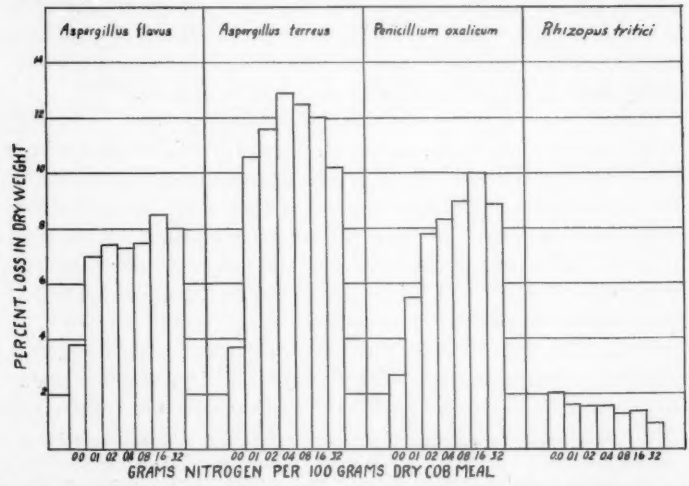

Aspergillus flavus
Aspergillus terreus
Penicillium oxalicum
Rhizopus tritici
PERCENT LOSS IN DRY WEIGHT
00 01 02 04 08 16 32
GRAMS NITROGEN PER 100 GRAMS DRY COB MEAL

FIG. 6. Influence of varying additions of mono-ammonium phosphate on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

gine, but additions of the nutrient covered a wider range—from none to 3.2 gm. nitrogen per 100 gm. of dry meal. The response of *Penicillium oxalicum* to ammonium phosphate was almost identical with its response to asparagine, and the optimum appeared to be near the same concentration of nitrogen. The two species of *Aspergillus* responded less vigorously to ammonium phosphate. With *A. flavus*, the optimum nitrogen addition, using the latter compound, appeared to approximate that for asparagine, but with *A. terreus* the optimum was apparently lower, about 0.4 or 0.4 to 0.8 gm. of nitrogen. With *Rhizopus tritici* a progressive decrease in dry-weight loss with the greater additions of nitrogen was found. This seems somewhat peculiar in view of the fact that with moderate additions of ammonium phosphate this fungus produced much more aerial mycelium in the early part of the growth period than where no nitrogen was added. The difference was still evident at the end of the period, though only very slightly. With the 3.2-gm. addition of nitrogen, no mycelial growth was apparent.

The effect of varying additions of calcium nitrate upon loss in dry weight is shown in figure 7. The experiment was similar to the preceding test with ammonium phosphate, except that additions of calcium nitrate ranged from none to 1.6 gm. nitrogen per 100 gm. of dry meal. As with ammonium phosphate, *R. tritici* showed decidedly negative results. In this case, however, the early mycelial growth, where only small amounts of nitrogen had been

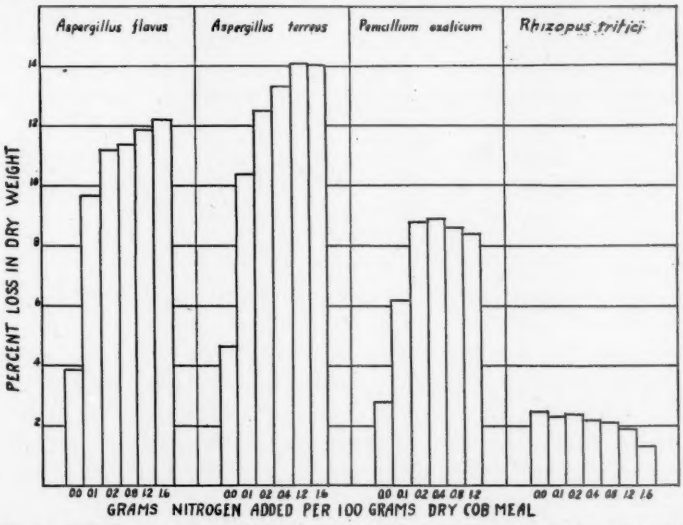


FIG. 7. Influence of varying additions of calcium nitrate on loss in dry weight of corncob meal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

added, showed but little, if any, increase in vigor, as compared with cultures with no added nitrogen; and, with the higher concentrations of nitrogen, growth was very weak throughout the experiment. *Aspergillus flavus* showed a response intermediate between that resulting from asparagine and from ammonium phosphate and here the optimum nitrogen addition was rather high, being about 1.6 gm. or above. *A. terreus* likewise showed an intermediate response, and the optimum nitrogen addition was approximately 1.2 to 1.6 gm. The response of *Penicillium oxalicum* was somewhat less than with either of the other two forms of nitrogen, and the optimum addition appeared to lie approximately between 0.2 and 0.8 gm. of nitrogen.

In comparing the effects of varying nitrogen additions upon thermogenesis and loss in dry weight of *Aspergillus flavus* cultures, it is notable that, whereas high additions of nitrogen—such as 1.2 to 1.6 gm. per 100 gm. of dry meal—gave greatest losses in dry weight where the fungus was allowed to grow for 28 days, considerably lower additions of nitrogen were sufficient to induce as rapid thermogenesis, during the peak of activity, as that obtained with larger quantities.

Influence of different forms of nitrogen added in equal quantities

In the work thus far reported only one form of nitrogen was used in each experiment. In order to compare the influence of different forms of nitrogen when added in equal quantities, two series of experiments were conducted, including four tests dealing with thermogenesis and one with loss in dry weight, in which all four organisms were employed. The following five compounds were compared:

Asparagine
Ammonium chloride (NH_4Cl)
Mono-ammonium phosphate ($NH_4H_2PO_4$)
Ammonium sulphate $(NH_4)_2SO_4$
Calcium nitrate $(Ca(NO_3)_2 \cdot 4\,H_2O)$

Each was used at the rate of 0.50 gm. of nitrogen per 100 gm. of dry meal.

Thermogenesis experiments.—In each of the four tests conducted relative to the influence of different forms of nitrogen upon thermogenesis, a different one of the four fungi was employed. Each experiment included one check and six cultures, one of which contained no added nitrogen. Each of the other five cultures was given an addition of one of the five compounds named above.

Figure 8 indicates the temperatures recorded in thermos-flask cultures of *A. flavus* throughout a period of 11 days. The striking feature of this experiment is the unusually high temperature of 49.25° C. reached by the

asparagine-containing cultures. Second to asparagine was calcium nitrate. The other cultures containing added nitrogen showed but slight differences, except for the ammonium-sulphate culture, which was noticeably lower. All

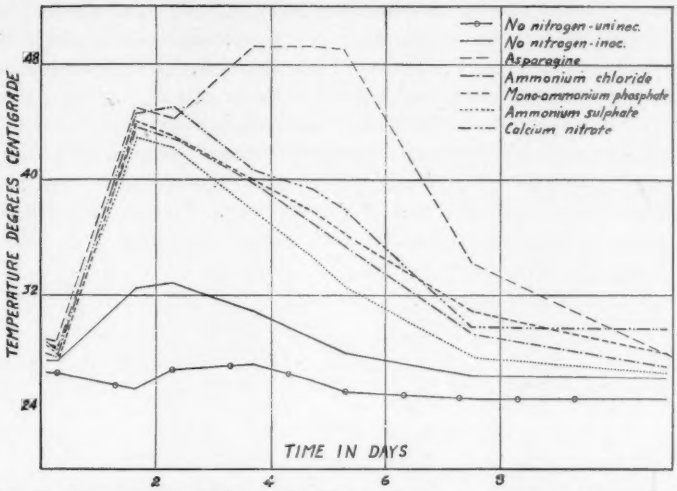


FIG. 8. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Aspergillus flavus*.

cultures supplied with nitrogen, in any form, showed temperatures far above those receiving no nitrogen addition.

The influence of different forms of nitrogen upon thermogenesis in cultures of *A. terreus* is shown in figure 9. Here, again, asparagine appeared to be superior to the other compounds, although the difference was much smaller than in the preceding experiment. The highest temperature re-

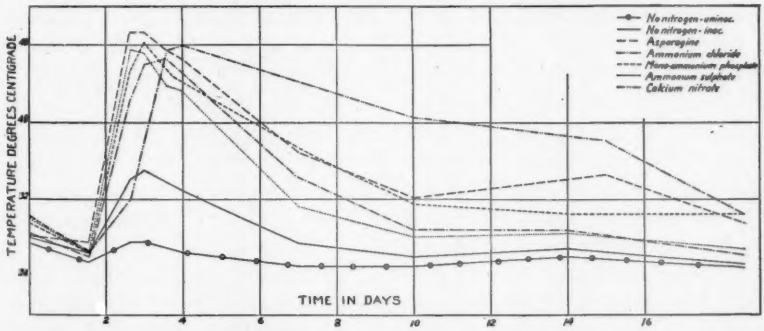


FIG. 9. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Aspergillus terreus*.

corded was 49.25° C., which was held for a period of at least six hours. Calcium nitrate, as with *A. flavus*, seemed to rank second, although this culture did not quite reach the temperature attained by the ammonium phosphate culture. Somewhat lower in thermogenesis, but still far above the no-nitrogen culture, were those supplied with ammonium chloride and ammonium sulphate. In general, then, the response of *A. terreus* to additions of different forms of nitrogen was much the same as that of *A. flavus*.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Penicillium oxalicum* are presented in figure 10. As usual, asparagine produced the most rapid response, and the temperature reached was higher than in all other cultures except one. The striking feature of

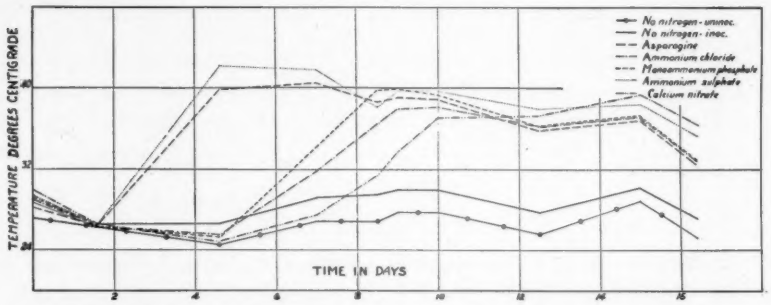


FIG. 10. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Penicillium oxalicum*.

this experiment is the marked superiority of ammonium sulphate, which produced a temperature of 42.25° C.—1.75° higher than that attained by the asparagine culture. It should be pointed out here that the delay of other cultures in beginning thermogenesis may not be significant in this experiment, from the standpoint of nutritional value of the nitrogen compounds to the growing cultures, since the beginning of apparent growth of *P. oxalicum* in loss-in-dry-weight experiments was frequently delayed several days, and in some instances did not occur at all. In this experiment, consequently, thermogenesis in certain cultures may have been brought about by the effects of the added salts in decreasing spore germination, rather than by any toxic effect upon, or by unavailability to, the fungous mycelia. If it so happened that initial growth was largely inhibited in parts of any cultures, then a slow rise in temperature might be attributed to heating in only a part of the culture at any one time. In such cultures the temperature peak would necessarily have been somewhat lower, though a longer period of thermogenesis could have been expected. This may have been so with calcium nitrate, for example, as shown in figure 10. It seems probable that, if the suitability of

the several compounds from the start were considered, asparagine and ammonium sulphate were the most favorable for thermogenesis of *P. oxalicum* in this experiment. As in the two preceding experiments, all forms of nitrogen resulted in very pronounced increases in thermogenesis.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Rhizopus tritici* are given in figure 11. In this experiment, contaminants were found in four cultures at the end of the ninth day, which cast some doubt upon the validity of the results. In three of the cultures, however, those receiving ammonium-chloride, -phosphate, and -sulphate, re-

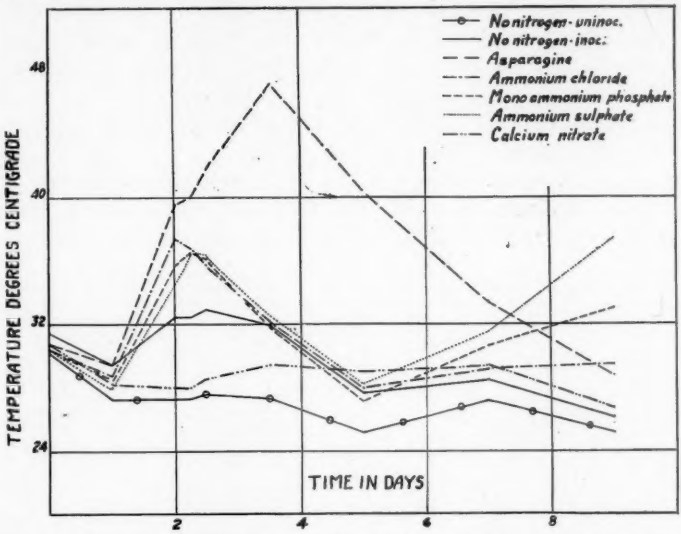


FIG. 11. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Rhizopus tritici*.

spectively, it seemed quite probable that no appreciable effect could have resulted from the presence of the contaminants before the third or fourth day at the earliest. This assumption is based upon the fact that the contaminants were fungi and were apparent in only a part of each of the cultures; further, it appears that the period of secondary heating by these organisms began somewhere between the fifth and sixth days. It is a matter of conjecture whether or not the bacterial contaminant in the asparagine culture had any appreciable influence upon the peak temperature which was reached during the fourth day. It is doubtful, however, if its effect was of any importance during the period when the other cultures were at their peak temperatures—about the end of, or shortly after, the second day.

If it may be assumed that the effect of contaminants was absent or negligible until after the first two and one-half days, then asparagine was apparently the most suitable source of nitrogen, with ammonium chloride ranking next. The effect of calcium nitrate was decidedly inhibitory, since the maximum rise in temperature—above the check—was 1.75° less in the calcium nitrate culture than in the culture containing no added nitrogen. The latter reached a temperature of 33.0° C.—5.25° above the check. This is the only case, in this series of four experiments in which the addition of a nitrogenous compound failed to result in a temperature considerably higher than that reached in the comparable no-nitrogen culture. It may be mentioned that, in the next experiment (fig. 12), dealing with loss in dry weight of cultures of these four fungi and comparing the same five compounds, the only culture which did not respond to nitrogen by producing more abundant apparent mycelial growth during the early part of the 28-day period was *R. tritici*, supplied with calcium nitrate.

Loss in dry weight.—As mentioned in the preceding paragraph, an experiment was conducted in which the influence, upon loss in dry weight, of additions of the five different forms of nitrogen previously tested was com-

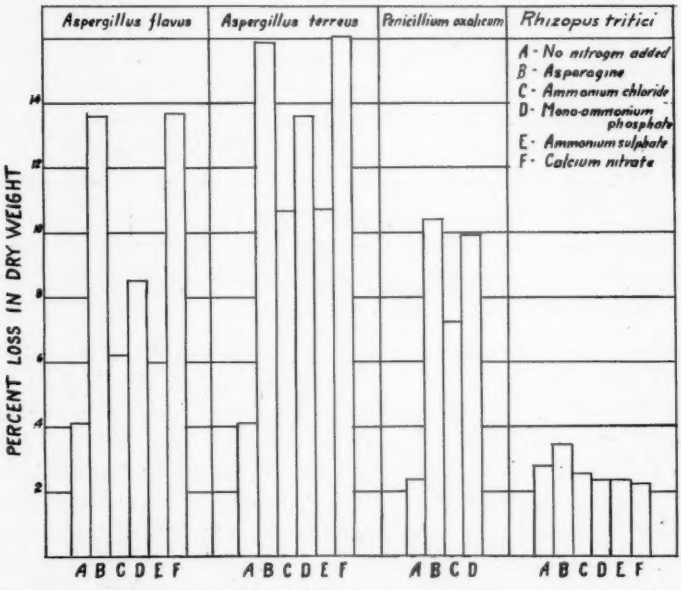


Fig. 12. Influence of additions of different forms of nitrogen on loss in dry weight of corncob meal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici*.

pared for the four fungi used in other experiments. The results are presented in figure 12.

The correlation, generally, between the results obtained in this and the four preceding experiments was noteworthy. For *Aspergillus flavus* and *A. terreus,* asparagine and calcium nitrate were distinctly superior to all other compounds used as sources of nitrogen, both for thermogenesis and loss-in-dry-weight cultures, and ammonium phosphate ranked next in both types of experiments.

Penicillium oxalicum produced no apparent growth in the samples of meal containing ammonium sulphate and calcium nitrate in the loss-in-dry-weight experiment (fig. 12). However, the distinct response to additions of asparagine and ammonium phosphate agreed with the results obtained in the thermogenesis experiment with this organism (fig. 10).

With *Rhizopus tritici,* the superiority of asparagine was quite distinct in both types of experiments. With the three ammonium salts, the effect upon dry-weight loss was inhibitory, whereas these compounds considerably increased thermogenesis. In the experiment with loss in dry weight, the apparent mycelial development was much greater during the early part of the 28-day growth period in cultures containing additions of these salts than in no-nitrogen cultures. At the end of the period, however, this difference had disappeared. It seems probable, then, that the presence of these compounds made possible more rapid development, as well as thermogenesis, during the first few days after inoculation, but that products resulting from their decomposition inhibited fungous activity after a longer period of growth, thus actually decreasing the total loss in dry weight in 28 days. Calcium nitrate resulted in the greatest reduction in dry-weight loss. As previously pointed out, it did not stimulate apparent mycelial growth even during the first part of the 28-day period, and correlated with this fact was its inhibiting effect upon thermogenesis.

Discussion

From the data presented it is apparent that, under the conditions of the experiments, nitrogen was an important factor in limiting both thermogenesis and loss in dry weight in cultures of the four fungi studied. Table I summarizes the results obtained from additions of five different compounds, respectively, to the corncob-meal substrate.

Examination of this table shows a rather close correlation between thermogenesis and loss in dry weight. For both species of *Aspergillus,* asparagine and calcium nitrate were outstanding in producing the highest thermogenesis as well as the largest losses in dry weight; ammonium sulphate and ammonium chloride were least effective in both types of tests; and monoammonium phosphate was intermediate.

TABLE I

SUMMARY OF THE RESULTS ON THERMOGENESIS AND LOSS-IN-DRY-WEIGHT OBTAINED IN CULTURES OF FOUR FUNGI GROWING ON VARIOUS SUBSTRATES

FORM OF NITROGEN ADDED	MAXIMUM RISE IN TEMPERATURE AND LOSS IN DRY WEIGHT							
	A. flavus		*A. terreus*		*P. oxalicum*		*R. tritici*	
	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.
	°C.	%	°C.	%	°C.	%	°C.	%
No nitrogen	7.00	4.01	7.25	4.01	3.00	2.38	5.25	2.82
Asparagine	23.50	13.59	21.75	15.91	15.25	10.39	19.75†	3.52
Ammonium chloride	18.25	6.22	19.50	10.66	10.50	7.25	10.00	2.49
Mono-ammonium phosphate	18.50	8.55	20.50	13.64	13.00	9.96	9.00	2.38
Ammonium sulphate	17.50	5.95	20.00	10.72	17.75	.....	9.00	2.38
Calcium nitrate	19.00	13.69	21.50	16.07	11.75	.....	3.50	2.27

* Influence of different forms of nitrogen on thermogenesis was tested in a separate experiment for each organism. The figures shown indicate maximum differences between checks and cultures.

† Maximum temperature may have been affected by a bacterial contaminant.

With *Penicillium oxalicum*, also, a correlation is shown by the partial data. The greatest amount of thermogenesis occurred in the culture containing ammonium sulphate, but unfortunately no loss-in-dry-weight data were obtained for this compound. Next in order in thermogenesis were the cultures containing additions of asparagine and mono-ammonium phosphate, respectively; and, as with the two species of *Aspergillus*, ammonium chloride was distinctly less effective. Likewise, among those loss-in-dry-weight cultures in which growth took place, asparagine gave the greatest response, mono-ammonium phosphate was second, and ammonium chloride third.

The results shown for *Rhizopus tritici* are of especial interest, in that only asparagine increased the loss in dry weight, whereas all forms of nitrogen used, except calcium nitrate, distinctly increased thermogenesis. An explanation of this seemingly peculiar occurrence has been proposed in connection with the presentation of the original data (figs. 11 and 12). It is sufficient to repeat here that all forms of nitrogen except the nitrate obviously stimulated early mycelial growth in the loss-in-dry-weight cultures, and it would seem that such a stimulation in the thermos-flask cultures was probably accompanied by more rapid decomposition of the substrate for a short period, and thus was responsible for the greater thermogenesis during this time. The accumulation of decomposition products of these compounds, however, was probably the cause for the decrease in the final loss in dry weight in 28 days—an effect not entering into the results appreciably during the short period in which the thermogenesis experiments were conducted. The dis-

tinct superiority of asparagine, as a source of nitrogen for *R. tritici*, is quite evident in its influence upon thermogenesis as well as upon loss in dry weight. As might be expected from the classification of molds by ROBBINS (15) calcium nitrate was not only unsuitable for this organism, but was decidedly inhibitory in its action. These results indicate an extension of the findings of ROBBINS (15) that *Aspergillus niger* has a higher apparent reducing intensity than *Rhizopus nigricans*. The data indicate that the same relation holds between *Rhizopus tritici* and *Aspergillus flavus*.

From the results set forth above it seems probable that the specific effect of each form of nitrogen in promoting thermogenesis depends directly upon the concomitant intensity of fungus decomposition of the substrate.

The influence of varying additions of three forms of nitrogen upon thermogenesis and loss in dry weight in cultures of *Aspergillus flavus* may be summarized as follows (table II).

TABLE II

THE INFLUENCE OF VARYING ADDITIONS OF THREE FORMS OF NITROGEN UPON THERMOGENESIS AND LOSS IN DRY WEIGHT IN CULTURES OF *Aspergillus flavus*

Nitrogen added per 100 gm. cob meal	Maximum rise in temperature and loss in dry weight					
	Asparagine*		Mono-ammonium phosphate*		Calcium nitrate*	
	Temp. rise†	Loss in dry wt.	Temp. rise†	Loss in dry wt.	Temp. rise†	Loss in dry wt.
gm.	°C.	%	°C.	%	°C.	%
0.00	8.00	3.59	6.50	3.80	5.50	3.89
0.01	7.00	.....	8.75	.....	7.25	.....
0.05	15.00	.....	16.25	.....	14.50	.....
0.10	17.75	8.43	20.50	7.00	22.50	9.72
0.20	.....	10.87	18.25	7.38	20.50	11.18
0.40	18.75	12.61	.....	7.32	22.25	11.02
0.80	.....	13.54	19.25	7.54	20.25	11.39
1.20	17.50‡	14.13	.....	.....	.....	11.93
1.60	.....	.....	18.75	8.52	21.25	12.21
3.20	.....	.....	13.25	8.08	9.50	.....

* Each form of nitrogen was tested in a separate experiment.
† Figures indicate maximum differences between checks and cultures.
‡ First peak in temperature is shown since second peak was probably affected by a bacterial contaminant.

In examining table II, the average maximum rise in temperature above the check for the no-nitrogen cultures was 6.67° C.; and for those cultures containing 0.01-, 0.05-, and 0.10-gm. additions of nitrogen it amounted to 7.67°, 15.25°, and 20.25° C., respectively. Above this point, increased additions gave results quite similar to that of 0.10 gm., except for the extremely large amount of 3.20 gm., which was obviously well beyond the optimum. With dry-weight loss, however, the results were somewhat different. With

each of the three forms of nitrogen, a progressive increase in percentage of loss in dry weight resulted from all the larger additions, except the extremely large amount of 3.20 gm.

Since the use of nitrogen in excess of 0.10 gm. per 100 gm. of meal did not materially increase thermogenesis—either in maximum temperature rise or in prolongation of heating—but did substantially increase loss in dry weight throughout a longer period (28 days), it might seem that small amounts of nitrogen were utilized by the fungus in the process of decomposition of the more readily available constituents of the corncob meal, during which time the most rapid development and respiration, together with greatest thermogenesis, took place. Following this period, with the aid of more nitrogen, the fungus was probably able to continue growth and respiration at a much less rapid rate, utilizing some of the more resistant carbohydrates present, and producing heat much more slowly. The more readily available food materials probably consisted principally of certain of the hemicelluloses, which comprise, according to BURKE (**2**) about 37 per cent. of corncobs, and the more resistant materials possibly included some of the true celluloses. This explanation is based largely upon the results of NORMAN (**12**), who found that the hemicelluloses were the constituents most rapidly lost in the early stages of decomposition of unsterilized oat straw, and who noted that this period corresponded with that of maximum thermogenesis in a vacuum flask containing similar material. The fact should not be overlooked that conditions for decomposition inside a mass of heating straw are decidedly different from those in straw which is not heating, though it seems doubtful whether this should greatly alter the sequence of decomposition.

The fact that the addition of a full nutrient combination was no more effective in stimulating thermogenesis of *A. flavus* than was an addition of the nitrogenous compound (calcium nitrate) alone, is of special interest, since it indicated that, so far as the food-nutrient composition of the substrate was concerned, nitrogen, or at most calcium nitrate, was the most important factor in thermogenesis in corncob-meal cultures of this fungus. Experiments in which other forms of nitrogen were employed demonstrated the fact that nitrogen, and not calcium, was the limiting factor.

Summary

1. The influence of various nitrogen additions, and certain other factors, upon thermogenesis and loss in dry weight of corncob-meal cultures of four species of fungi was studied.

2. The organisms employed—*Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici*—had previously been isolated from alfalfa hay, and found to be capable of considerable heat production in moist hay cultures.

3. Five forms of nitrogen—asparagine, ammonium chloride, mono-ammonium phosphate, ammonium sulphate and calcium nitrate — each greatly stimulated both thermogenesis and loss in dry weight when added to the substrate in cultures of *Aspergillus flavus, A. terreus,* and *Penicillium oxalicum.* With *Rhizopus tritici,* all forms of nitrogen markedly increased thermogenesis, with the exception of the nitrate form which showed a distinct inhibiting effect. Only asparagine increased dry-weight loss in cultures of this organism, all other compounds showing a depressing influence, with the nitrate being most effective in this regard.

4. Asparagine was found to be most generally suitable for the four fungi employed, both in its effect upon thermogenesis and upon loss in dry weight. The highest temperature recorded throughout the experiments—49.25° C.—was reached in cultures of *Aspergillus flavus* and *A. terreus,* each of which had been supplied with asparagine. The significance of these figures is shown by the fact that the maximum temperature above the check in both instances exceeded 21.50° C., as compared with cultures containing no added nitrogen, in which the temperatures reached were not more than 7.25° C. above the check with either fungus. The greatest loss in dry weight, in 28 days, occurring throughout the course of the experiments, was 16.95 per cent., which was found in cultures of *A. terreus* supplied with asparagine, and may be compared with 3.54 per cent. loss occurring at the same time in cultures of this organism containing no added nitrogen.

5. Nitrogen additions ranging from 0.01 to 0.10 gm. per 100 gm. of dry cob-meal generally resulted in progressive increases in thermogenesis in cultures of *A. flavus.* The average maximum rise in temperature above the check, for additions of three forms of nitrogen—asparagine, mono-ammonium phosphate, and calcium nitrate—at the rate of 0.00, 0.01, 0.05, and 0.10 gm. per 100 gm. of meal, were 6.67°, 7.67°, 15.25° and 20.25° C., respectively. Greater additions, ranging from 0.20 to 1.60 gm., inclusive, generally gave results similar to that shown for the 0.10-gm. addition, with an average of 19.64° maximum rise in temperature; the use of 3.20 gm. nitrogen, as mono-ammonium phosphate and calcium nitrate, resulted in an average maximum rise of only 11.38°.

6. Progressive increases in dry-weight loss in cultures of *A. flavus* followed the larger additions of asparagine, mono-ammonium phosphate and calcium nitrate, respectively, up to 1.2 and 1.6 gm. nitrogen per 100 gm. of dry cob-meal. The other three organisms differed considerably in response to various amounts of these compounds.

BOTANY AND PLANT PATHOLOGY SECTION
IOWA AGRICULTURAL EXPERIMENT STATION
AMES, IOWA

LITERATURE CITED

1. BARTHEL, C., and BENGTSSON, N. Action of stable manure in the decomposition of cellulose in tilled soil. Soil Sci. **18**: 185–200. 1924.
2. BURKE, GEORGE W. I. Some analytical data on corn cobs and their parts. II. The furfural yield and the pentosan content of cobs and their parts. III. Comparison of phloroglucinol and thiobarbituric acid as precipitants for furfural. M. S. thesis in Chemical Engineering, Iowa State College, 40 p. Unpublished. 1923.
3. GILMAN, J. C., and BARRON, D. H. Effect of molds on temperature of stored grain. Plant Physiol. **5**: 565–573. 1930.
4. HARRISON, J. W. Thermogenesis in hay-inhabiting fungi. Iowa State College Jour. Sci. **9**: 37–60. 1934.
5. HENRY, W. A., and MORRISON, F. B. Feeds and feeding. The Henry-Morrison Co., Madison, Wisconsin, pp. 4–12, 153–711. 1927.
6. HEUKELEKIAN, H., and WAKSMAN, S. A. Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi. Jour. Biol. Chem. **66**: 323–342. 1925.
7. JAMES, L. H. Microbial thermogenesis. Doctoral Dissertation, Yale University. Unpublished. 1927.
8. ————. Studies in microbial thermogenesis. I. Apparatus. Science n.s. **65**: 504–506. 1927.
9. ————, RETTGER, L. F., and THOM, C. Microbial thermogenesis. II. Heat production in moist organic materials with special reference to the part played by microörganisms. Jour. Bact. **15**: 117–141. 1928.
10. MIEHE, HUGO. Die Selbsterhitzung des Heus. Eine biologische Studie. 127 pp. G. Fischer. Jena, 1907.
11. ————. Die Wärmebildung von Reinkulturen im Hinblick auf die Aetiologie der Selbsterhitzung pflanzlicher Stoffe. Archiv. Mikrobiol. **1**: 78–118. 1929.
12. NORMAN, A. G. The biological decomposition of plant materials. Part III. Physiological studies on some cellulose-decomposing fungi. Ann. Appl. Biol. **17**: 575–613. 1930.
13. ————. The biological decomposition of plant materials. Part IV. The biochemical activities on straws of some cellulose-decomposing fungi. Ann. Appl. Biol. **18**: 244–259. 1931.
14. PHILLIPS, MAX. The chemistry of lignin. III. The destructive distillation of lignin from corn cobs. Jour. Amer. Chem. Soc. **51**: 2420–2426. 1929.
15. ROBBINS, W. J. The assimilation by plants of various forms of nitrogen. Amer. Jour. Bot. **24**: 243–250. 1937.

16. SWEENEY, O. R. The commercial utilization of corncobs. Iowa State College Eng. Exp. Sta. Bull. **73**. 1924.

17. WAKSMAN, S. A. Cellulose and its decomposition in the soil by microorganisms. Internatl. Rev. Sci. and Pract. Agr. n.s. **4**: 759–770. 1926.

18. ———— and DIEHM, R. A. On the decomposition of hemicelluloses by microörganisms: II. Decomposition of hemicelluloses by fungi and actinomyces. Soil Sci. **32**: 97–117. 1931.

TRANSLOCATION OF CARBOHYDRATES IN THE SUGAR BEET[1]

O. A. LEONARD

(WITH EIGHT FIGURES)

Introduction

This paper covers certain physiological phases of a series of investigations on the production of sugar beets in Iowa, and is intended to aid in the interpretation of pathological and cultural studies. In a plant whose commercial importance depends upon its sugar content, problems of photosynthesis, translocation, transformation, and storage obviously are important. The relation of the four principal carbohydrates of the sugar beet, sucrose, fructose, glucose, and dextrin to these processes has been the object of this investigation.

Literature review

Literature dealing with the sugars in the leaves and roots of the sugar beet is voluminous, but only a small fraction of the work is related to photosynthesis, translocation, transformation, and storage. In this review, only that sugar beet work which has a particular bearing on the present problem will be referred to and it will be discussed in chronological order.

GIRARD (9) in his studies on the chemical composition of leaves and roots of sugar beets, found that sucrose in leaves fluctuated more between day and night than did the reducing sugars. At evening sucrose concentration was sometimes observed to be twice as great as it was the next morning, while reducing sugars sometimes remained constant. He concluded that sucrose is the first product of photosynthesis and is moved to the root as such. Both midribs and blades, however, were analyzed together. The high percentage of reducing sugars in the midribs undoubtedly masked diurnal variations which occurred in the blades. He was unable to detect reducing sugars in roots. MACQUENNE (13) contrary to GIRARD, believed that the reducing sugars were the first products of photosynthesis and that they migrated to the roots in that form, and were there condensed into sucrose.

LINDET (11) investigated the proportion of glucose to fructose in different parts of the leaves. In the early stages of growth (July) glucose usually exceeded fructose in the blades, while in the petioles, fructose was found to be low in comparison to glucose. He believed that the excess of fructose in the leaves was due to glucose being consumed in the tissues by respiration more rapidly than fructose. Low fructose values for the petioles

[1] Journal Paper no. J–580 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 449.

were believed to be due to fructose being used more easily than glucose in the formation of new tissues.

STRAKOSCH (21), employing microchemical methods, thought glucose to be the only sugar in the mesophyll cells of the sugar beet. Migration of glucose into the veins was followed by the appearance of fructose therein and later by the formation of sucrose. He believed that glucose was the first product of photosynthesis and that fructose was formed from it. In the veins and petioles, glucose and fructose were condensed to form sucrose and translocated to the roots in that form. PEKLO (16), also from microchemical data, concluded that the sieve tubes contained the greatest amount of sucrose.

ROBERTSON, IRVINE, and DOBSON (18) studied the distribution of enzymes in the blades, petioles, and roots of the sugar beet. They found that invertase was present in blades and petioles but was absent from the roots. They believed that invertase synthesized as well as hydrolyzed sucrose and, therefore, concluded that sugars were translocated to the root in the form of sucrose. STEPHANI (20) supported this view.

RUHLAND (19) held that, in the sugar beet, sugar migrates in the leaves not as sucrose, but as reducing sugars and perhaps mainly as fructose. Reducing sugars were considered to be condensed into sucrose upon entering the roots where they are stored and from whence they cannot be moved until drawn upon by growth. Reducing sugars at times actually decreased from the apex to the base of the petioles. Cells of the leaves were said to be permeable to fructose, glucose, sucrose, and more or less to all the sugars tested. Starch was formed in the leaves when any of these sugars was supplied externally.

CAMPBELL (4), working on the mangold plant which, like the sugar beet, is a variety of *Beta vulgaris* L., suggested that reducing sugars were the first carbohydrates to be formed as soon as daylight began. A little later the sucrose curve began to rise, and later still the "starch" curve. The sucrose curve did not appear to rise before the reducing sugars had reached their maximum, which they maintained throughout the period of illumination. Similarly, the "starch" did not seem to rise until sucrose had reached its maximum. Young leaves had a much higher level of reducing sugars than old leaves, but sucrose content was about the same. The fraction reported as "starch" was probably dextrin rather than starch.

In 1916, DAVIS, DAISH, and SAWYER (6) published the most complete work which had yet been done on *Beta vulgaris* L. Some of their conclusions were: (1) Starch was absent from the leaves, except in the very early stages of growth before the root had undergone appreciable enlargement. (2) Maltose was absent at all stages of growth. (3) In the midribs and petioles the hexoses were always greatly in excess of sucrose and varied greatly between

day and night and throughout the season, whereas sucrose remained relatively constant. The ratio of the hexoses to sucrose increased from the blade to the base of the petiole. As the season progressed, the predominance of the hexoses in the blades, midribs, and petioles became more marked. (4) Sucrose was assumed to be the first sugar of photosynthesis. It was hydrolyzed, they said, and moved to the root in the form of invert sugar. Once in the root it was again converted into sucrose and remained there until it was used for growth of the following year. (5) Sucrose is probably not synthesized by invertase.

TOTTINGHAM, *et al.* (**22**) have published data on the effects of climate on the sugar beet. They noted that temperatures of 30° C. limited photosynthesis. Fluctuations in reducing sugars and sucrose were observed during the day. Sucrose was frequently absent from the petiole, and less often from the blade. Reducing sugars were always present in both blade and petiole.

DOBY and HIBBARD (**7**) investigated the effect of nutrient ions upon enzyme activity in the sugar beet. They concluded that the quantity of enzyme is controlled by the type of nutrition, being greater in plants growing in a potassium-deficient solution than in those in a complete nutrient solution. The NO_3 ion increased the invertase activity, but the Cl ion inhibited it. Greatest invertase activity was found in young leaves.

BULGAKOVA, *et al.* (**3**) reported on diurnal fluctuations and gradients in sugars in the blades and petioles of sugar beets. They found that plants grown under a combination of electric lights and sunlight showed maximum reducing sugars in the petiole, slightly less in the veins, still less in the leaf parenchyma, and least in the roots. Sucrose increased continuously from leaf parenchyma to roots. Reducing sugars in the leaf parenchyma increased and reached their maximum towards evening. In the veins and petioles reducing sugars reached their greatest concentration in the first half of the day and decreased in the second. A close correlation between variations in the percentages of reducing sugars in the parenchyma, and the strength and duration of light suggested to these authors that reducing sugars were the first products of photosynthesis and that they were translocated to the root in that form.

BENNETT (**2**) observed that the curly top virus may move at rates up to 60 cm. per hour through sugar beet plants and suggests that this indicates a rapid movement of organic food materials.

Materials and methods

Most of the sugar beets used in this study were grown on the Northern Iowa Experimental Association farm at Kanawha, Iowa, but some were grown in the greenhouses at Ames, Iowa. In 1935 the U. S. no. 1 variety

was used and in 1936, the Pioneer variety. The plants grown on the experimental farm were spaced at 12-inch intervals in rows 22 inches apart.

Care was exercised to obtain comparable samples for chemical analyses. Border rows were not used and all collections were taken from a restricted portion of a commercial field to eliminate as much as possible the effects of soil variability. Fifteen to 20 entire beet plants were pulled and brought directly to the laboratory. One or two recently matured leaves were removed from each beet plant, the blades separated from the midribs, and 100-gm. samples of the blades weighed to $\pm$ 0.10 gm. The samples were then placed in one-quart Mason fruit jars containing 450 cc. of boiling 95 per cent. redistilled ethyl alcohol and boiled for 15 minutes. The samples were stored in the dark until analyzed, which was usually within a few weeks.

Petioles were divided into three parts: (1) The "upper" petiole consisting of the lower part of the midrib and the upper inch of the petiole proper, (2) the "middle" petiole extending from the "upper" petiole to within 3 or 4 inches of the base, and (3) the "lower" section consisting of the last 3 or 4 inches of the petiole. Only the central part of the "middle" petiole was used in all collections. The term "petiole" in the diurnal studies refers to the middle petiole section. Duplicate 75- to 100-gm. samples were weighed as before, sliced into 3- to 5-mm. pieces with a sharp knife, dropped at once into jars containing 450 cc. of boiling 95 per cent. alcohol, simmered for 20 minutes and then stored as before.

Roots were washed free from earth, and the corky layer removed. Thin transverse slices were obtained from the central part of each root. Duplicate 100-gm. samples of these slices, composited from 15 to 20 beets, were weighed as before, killed in 450 cc. of boiling 95 per cent. alcohol, allowed to simmer for 15 minutes, and then stored.

Greenhouse samples of the blades, petioles, and roots were considerably smaller than field samples. These were usually killed in 250-cc. Erlenmeyer flasks, containing 100 to 150 cc. of boiling 95 per cent. alcohol and treated as above.

Experiments on the reversal of translocation

All experiments on the reversal of translocation were performed in the greenhouse. Three-months-old sugar beet plants and mother sugar beets transplanted from the field were placed in the dark room. Samples were taken at once, and at various intervals while in the dark. Samples of mature and young leaves were collected from the three-months-old plants, while only mature leaves were collected from the mother beets. Petioles were divided as in the diurnal series, except that the term "upper" petiole included both the "middle" and "upper" petiole sections. After the

three-months-old beet plants had been in the dark for a period of time, they were transferred to the light. Some of the mature leaves were bagged, using two large paper bags on each leaf, while others were left uncovered and harvested after several days. The mother sugar beet plants were left in the darkroom for a longer period of time than the three-months-old plants in order to obtain a sample of etiolated leaves. These were collected and divided in the usual manner.

The samples were of various sizes and were killed in Erlenmeyer flasks, containing boiling 95 per cent. alcohol, allowed to simmer for 10 or 15 minutes, corked, and stored for analyses.

Methods of chemical analyses

The preserved material was transferred to 400-cc. beakers and usually two extractions with boiling 80 per cent. alcohol were made per day until the reducing substances had been removed from the samples as determined by tests. From 15 to 20 extractions were required. Extracts were made up to 1 liter for aliquoting.

Methods of carbohydrate analyses described by Loomis and Shull (**12**) were used in this investigation. Reducing sugars and sucrose were determined by the Munson-Walker-Bertrand method, fructose by Jackson and Mathews' modification of Nyn's method, and dextrin was extracted in 10 per cent. alcohol. Sucrose was inverted by invertase.

Experimental data

Microchemical studies

Blades, petioles, and roots were studied for the presence and location of reducing sugars, sucrose, and starch. In general the tests for sucrose were not good. The Flückiger reaction was used for testing for sugars and the alcoholic IKI test for starch.

Reducing substances were found to occur in slight amounts in leaf parenchyma, but were more concentrated along the veins and in the guard cells. In the mesophyll there were sometimes a few bodies which stained with IKI.

In the petioles, longitudinal sections were more serviceable than transverse sections for determining the localization of sugars. Reducing substances were most concentrated in the parenchyma, less in the xylem and phloem, and least in the bundle cap. Sucrose was more concentrated within the veins. This relationship was also shown by macrochemical tests on dissected tissue. Starch occurred as minute grains in the starch sheath over the bundle caps and in starch-containing cells which sometimes surrounded the entire vein. These minute starch grains or bodies which stained dark with IKI were always present.

Roots contained only a small amount of reducing substances, which were most concentrated towards the periphery. Starch was not found.

Carbohydrate changes with photosynthesis

Girard (9) found only slight diurnal variations of reducing sugars in the leaves of sugar beets, while variations in sucrose were marked. In the present investigation midribs and petioles were removed from the lamina, so that variations represent more closely the fluctuations within the photosynthetic mesophyll cells. Figures 1 and 2 show that diurnal variations in

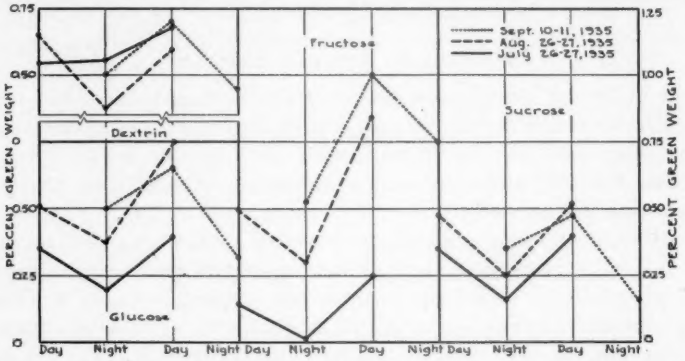


Fig. 1. Percentage and kinds of carbohydrates in leaf blades at different times during the season.

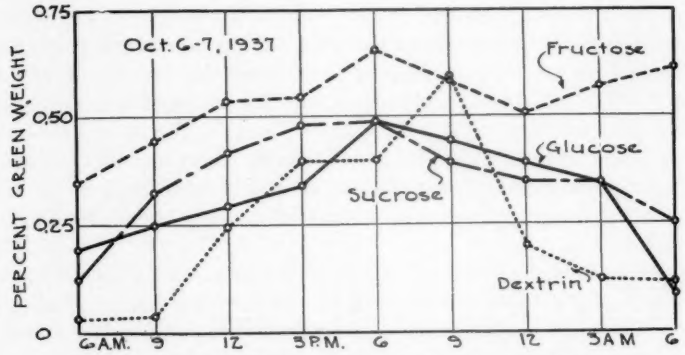


Fig. 2. Diurnal variations of carbohydrates in leaf blades.

glucose and fructose were equal to those of sucrose. Similar results were obtained in other collections, the data from which are omitted to conserve space. In figure 2 it may be observed that the sucrose curve rises more sharply in the morning than do the curves for glucose and fructose. How-

ever, in two other collections the glucose and fructose curves rose as sharply or even more so than the sucrose curves; hence one cannot tell from their early morning behavior which one, or ones, of these sugars is the first product of photosynthesis. Sucrose is not necessarily the first product of photosynthesis, as GIRARD believed (9), if the fluctuations in sucrose, as compared with the fluctuations of the hexoses, are taken as criteria.

It will be shown later that reducing sugar and sucrose levels in blades are governed by the relative activities of a sucrose-synthesizing system and invertase activity. If invertase activity is relatively low and the synthesizing system active, we may find, as in corn, mainly sucrose in the leaves; whereas, if invertase is active in the leaves, even though a sucrose-synthesizing system exists, as in the sugar beet, the sugars may be present mainly in the inverted state. This relationship is, likewise, correlated with the percentage of water in the tissue, as has been shown by the writer in work done on the sunflower (10). These relationships may explain the behavior of different plants in fluctuations of sucrose and reducing sugars during the day. It is easily conceivable that a sugar could be the first product of photosynthesis and still not fluctuate greatly in the blades. Complete absence of a sugar during periods when photosynthesis is in progress might be taken, however, as evidence that this sugar is not being formed. CLEMENTS (5), on this basis, has eliminated mannose as a product of photosynthesis.

The percentage of fructose relative to glucose increased as the season advanced (fig. 1). The response of glucose and fructose to photosynthesis did not differ greatly, however, at different seasons. It is apparent that "*static*" portions of sugar are present within the leaves. The *static* portions of glucose and sucrose increased only slightly as the season advanced, while that of fructose increased from none in July to a point higher than either glucose or sucrose in September. This accumulation of fructose may mean either that it was used more in growth early in the season, or that the glucose-fructose equilibrium shifted as the sugars accumulated. It may be seen from figure 1 that glucose was in excess of fructose in July. In September, fructose was in excess of glucose, and the "static" fraction was considerably greater than in July. Growth had slowed down at this time, and the beets were maturing. Young beet plants grown in the greenhouse were always found to be low in fructose. It seems that there is a distinct correlation between growth and low fructose levels within leaves. Growth was always associated, however, with a decrease in the total sugar concentration within the leaves, and this reduction may affect the glucose-fructose equilibrium. It will be shown later that fructose and glucose are interconvertible in sugar beet leaves.

Dextrin accumulated under certain conditions. Generally, young beet plants grown in the greenhouse contained a very small percentage of dex-

trin. In some field experiments dextrin was low and showed no tendency to accumulate until toward the end of the daylight hours. This condition was observed during periods when growth was rapid and sugar percentages were low. In later collections (figs. 1 and 2) dextrin showed a pronounced diurnal variation, perhaps because of the greater sugar concentration.

TRANSLOCATION

NORMAL TRANSPORT.—The three sugars, glucose, fructose, and sucrose fluctuated in the blades during the day and were often associated with similar variations in the petioles. Dextrin fluctuated in blades, but these changes were associated with no marked reactions in petioles. These results suggest that dextrin is largely hydrolyzed to glucose before it is translocated; hence the following discussion will deal more with the three sugars mentioned than with dextrin.

Figures 3 to 5 show that all of the sugars exhibited diurnal variations in the petioles. There was a large static portion of sugar within the petioles which somewhat masked variations which occurred. Table I and figure 5

TABLE I

PERCENTAGE OF THE SUGARS AND DEXTRIN IN BLADES, PETIOLES, AND ROOTS OF FIELD-GROWN SUGAR BEETS ON JULY 26–27, 1935. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PLANT PART	TIME OF COLLECTION	GLUCOSE	FRUCTOSE	SUCROSE	DEXTRIN
		%	%	%	%
Blades	3 P.M.	0.35	0.15	0.35	0.55
	4 A.M.	0.20	trace	0.15	0.56
	2 P.M.	0.40	0.25	0.40	0.67
Petiole	3 P.M.	2.30	0.48	0.45	0.35
	4 A.M.	1.88	0.38	0.30	0.37
	2 P.M.	2.50	0.48	0.45	0.35
Root	3 P.M.	0.16	0.00	9.20	.....
	4 A.M.	0.15	0.00	8.99	.....
	2 P.M.	0.15	0.00	9.95	.....

show, however, that there were diurnal variations which were as much as 0.5 per cent. of the green weight of the tissue.

From cross-sectional measurments of the sieve tubes it was estimated that they occupy less than 0.25 per cent. of the volume of the petiole. It is evident that even though the sieve tube contents were to fluctuate between 0 per cent. of sugar at night and saturation in the afternoon, the phloem could not have held all of the sugar observed to accumulate within the petiole. Microchemical tests always showed that reducing sugars were more concentrated in the surrounding parenchyma than in the phloem. The

writer concludes, therefore, that there was an exchange of sugars between the phloem and the surrounding cells. It is not certain that the sugars which appeared to fluctuate within the petiole were actually the sugars

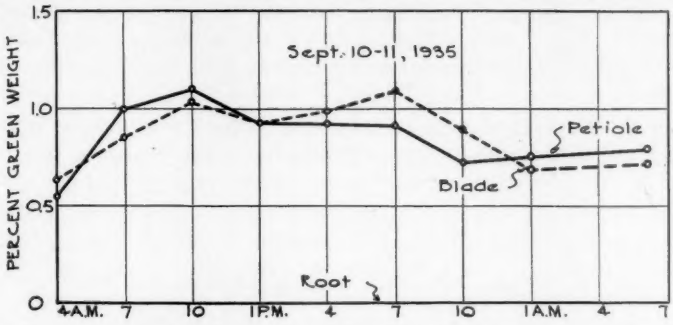


FIG. 3. Diurnal variations in fructose of blades, petioles, and roots.

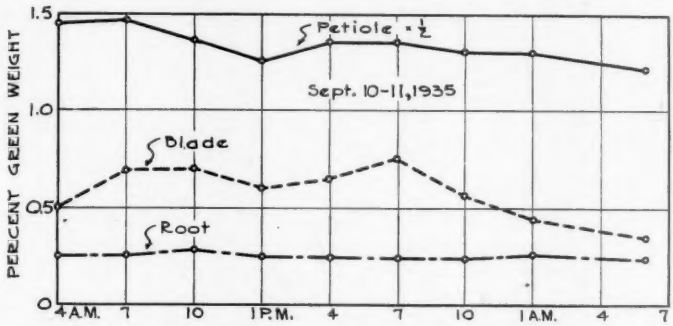


FIG. 4. Diurnal variations in glucose of blades, petioles, and roots.

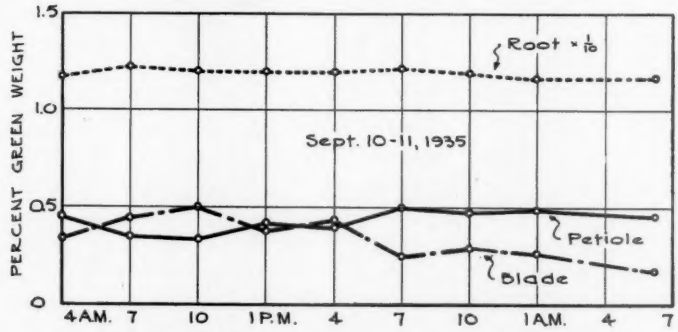


FIG. 5. Dirunal variations in sucrose of blades, petioles, and roots.

being moved. If it were not for sugar leakage from sieve tubes, there should have been no noticeable diurnal variations within the petioles. In passing from sieve tubes to the surrounding cells, sugars came into contact with various hydrolyzing, synthesizing, and transforming systems, and fluctuations of a given sugar within the entire petioles cannot be taken as evidence that this sugar was being transported in the sieve tubes of the petioles.

A close correlation between fluctuations of a sugar in the blades and in petioles probably indicates translocation, and could hardly be expected to

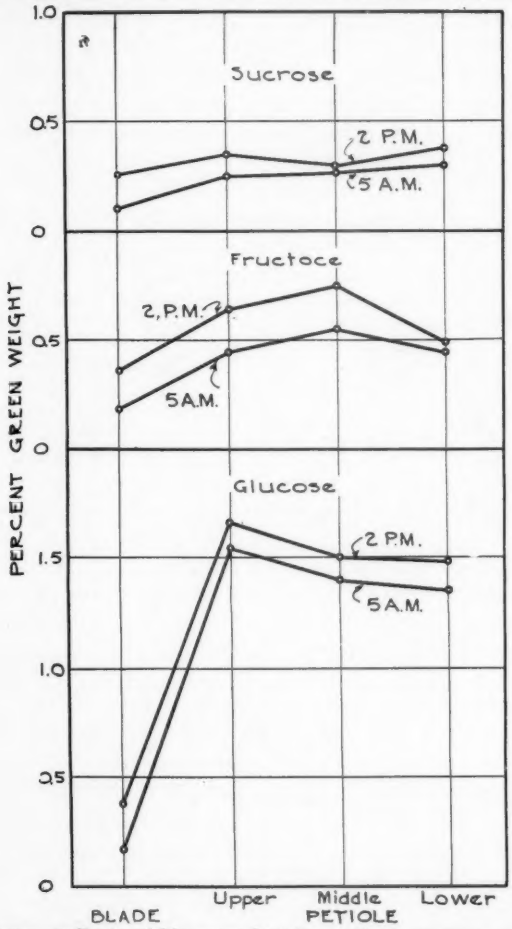


FIG. 6. Sugar gradients within sugar beet leaves in the morning and in the afternoon, September 9, 1936.

occur on the basis of chance. Such correlation in the fluctuations of fructose in blades and petioles is shown in figure 3 and it is believed to indicate fructose translocation. ENGARD (**8**), from ringing experiments on the red raspberry, was able to demonstrate, microchemically, that reducing sugars accumulated in the phloem above a ring and decreased to a low level below it. Cuprous oxide crystals formed in the test were localized in the phloem with only a slight precipitate in the surrounding cells. This experiment indicates that the phloem translocated reducing sugars. MASON and MASKELL (**14**) have shown that sucrose is a moving form in cotton. On the basis of this and other literature, and of the data presented here, it seems likely that all three sugars are translocation forms.

Figure 6 shows that all three of these sugars increased during the day and decreased by night in all parts of the petioles. Other and similar data were collected. These data strengthen the preceding in showing that sugars actually fluctuate in the petioles. There apparently is no polarization in the petiole between the phloem and the surrounding cells. During the day the concentration of the sugars increased in the phloem, this increase being followed by an increase in the surrounding cells. At night the concentration of sugars in the phloem decreased and this decrease was followed by a reversal of sugar movement.

POLAR TRANSPORT.—Two problems on polarity were tested. These were: (1) Do the sugars migrate out of the leaf mesophyll into the phloem in response to stimuli other than the lower sugar concentration of the latter tissue? (2) Is there, existing within the phloem of the petiole, a polarity which induces sugars to accumulate at the base of this organ? In other words, do sugars move in response to a polar condition existing within the phloem, or simply along diffusion gradients which keep the phloem filled with sugar as rapidly as it is removed into the sugar-storing cells?

Leaves, detached at the base of the petioles, were placed in beakers of water and removed to the darkroom. Table II shows a decided decrease in all three sugars in the blades. This decrease was shown to be due to respiration and movement of sugars from the blade into the petioles of the detached leaves. This experiment was repeated four times and in all cases similar results were obtained.

The transport of sugars from the blades was not sufficient to produce any marked effect within the petiole. It is significant, however, that within the petiole there was no redistribution of sugar, and no evidence of polar accumulation of sugars at the base of the petiole. The sugars must have been in a state of relatively stable equilibrium, since there was no evidence of transformation of one sugar into another, nor of synthesis or hydrolysis.

Another experiment was performed to check the above conclusions. Leaves of sugar beets were detached, placed in quart jars filled with water,

TABLE II

LOSSES OF SUGARS FROM BEET LEAVES AFTER BEING DETACHED FROM PLANTS AND PLACED IN DARK FOR 24 HOURS. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

LEAVES	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
Attached leaves (At beginning of experiment)			
Blade	0.32	0.57	0.36
Upper-petiole	2.26	0.95	0.67
Middle-petiole	2.25	1.00	0.60
Lower-petiole	2.20	0.81	0.61
Detached leaves (After 24 hours in the dark)			
Blade	0.11	0.38	0.12
Upper-petiole	2.23	0.90	0.65
Middle-petiole	2.23	1.00	0.57
Lower-petiole	2.20	0.83	0.55

and exposed to diffuse sunlight. Figure 7 shows that movement of the sugars continued, despite the fact that the leaves were detached from the plants. It is evident that the sugars moved from a region of low concentration in the blades to one of high concentration in the midribs and petioles. This experiment was repeated three times and in all cases similar results were obtained. It is of interest to note that there was no greater tendency for sugars to accumulate in one part of the petiole than in another. It seems probable that sugar was maintained at a uniform level throughout the phloem system of both leaf and petiole and that no polarity existed within the phloem system itself. The active tissue in polar transport would then become the border parenchyma of the leaf veinlets.

Dextrin showed practically no fluctuations in the petioles in the diurnal series, nor did it accumulate in the petioles of detached leaves placed in the sun as much as the sugars (fig. 7) did. Probably it was mainly hydrolyzed before it was moved.

REVERSAL EXPERIMENTS.—Young beet plants, about three months old, were placed in a darkroom. Collections of mature and young leaves were taken at once and after periods of storage. It was found that there was at first some removal from the mature leaves, but after three days the slight losses were probably caused by respiration. After five days the plants were transferred to the sunlight and some of the leaves were bagged. There was an increase in the sugars in the blades and petioles of the leaves left in the sun, but there was no increase in the bagged leaves. If there was any reversal of the translocation from the illuminated to the darkened leaves or from the roots to the darkened leaves, it was not measurable. The plants were again transferred to the darkroom. By this time the sugars had increased to the

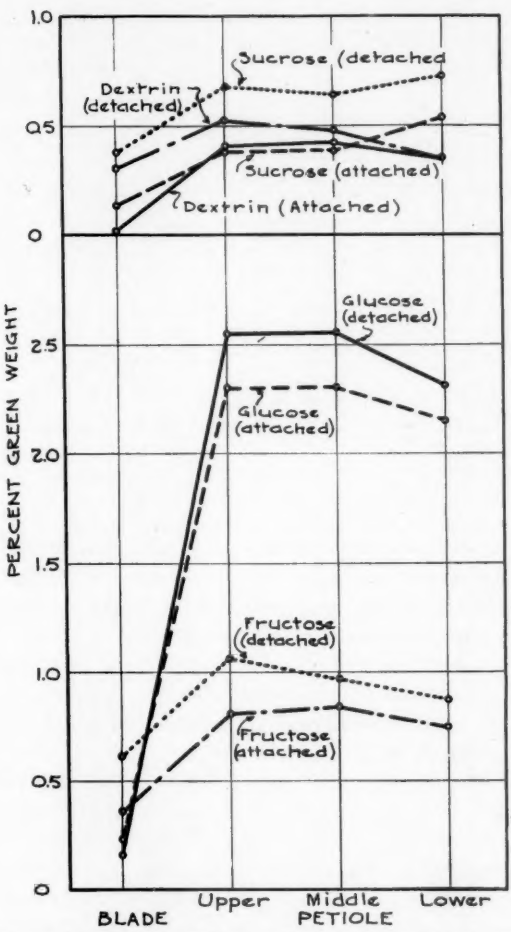


FIG. 7. Changes in the carbohydrates of detached leaves placed with their petioles in water and left in sunlight for 11 hours.

point where again there was translocation. The plants were shifted again to the light, with some of the leaves bagged (fig. 8). There was a loss of sugars from the bagged leaves, indicating no reversal of the translocatory stream. By ringing cotton plants and bagging some of the leaves, PHILLIS and MASON (17) were able to reverse the translocatory stream in leaf veins but reversal into the mesophyll was doubtful. Under their conditions the "sink" was removed. The "sink" or normal storage organ was not removed in the sugar beet experiments.

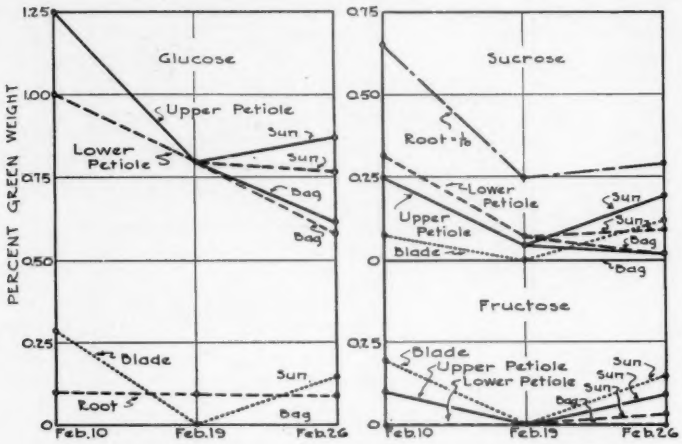


FIG. 8. Variations in the sugars of fully grown leaves after the plants were placed in the dark and then transferred to the light with some leaves bagged.

It is of interest to observe how low the concentration of the sugars in the mesophyll may drop and still have no backflow from the veins. We may see from figure 8 that the sugars were removed until they were not detected in the blades, although the concentration in the petioles was still high. Evidently the polarizing force which prevents the sugars from moving back into the mesophyll through the phloem is very strong.

Young leaves were collected and analyzed, as were the old. They were marked with India ink when placed in the dark and thus comparable leaves were collected. During the experiment the leaves more than doubled in size, yet their sugar percentage did not decrease greatly. Evidently a considerable quantity of sugar was translocated from the roots into these young leaves which is a contrast when compared with the behavior of the mature leaves, and suggests that the mechanism of polarization is not developed until late in the growth period of the leaf.

Fructose decreased to zero in all parts of the young leaf. The roots contained mainly sucrose; hence most of the sugars resulting from the reversal of translocation arose from sucrose. When sucrose is hydrolyzed by invertase, it yields equal amounts of fructose and glucose. Evidently the fructose which was formed from the hydrolysis of sucrose was either transformed into glucose or used rapidly in growth. It will be shown later that blades floated on a fructose solution converted some of the absorbed fructose into glucose. Whenever growth is rapid, fructose appears to be low, and there is evidence that this sugar may be used for synthesis to a greater extent than glucose.

Perhaps both transformation of fructose to glucose and a more rapid use of fructose in growth took place at the same time.

Sucrose was low in the young leaves and did not increase in concentration, even though it may be assumed to have moved in from the roots. Invertase is very active in young blades and petioles and probably tends to keep the sucrose in the inverted state.

GRADIENTS IN ETIOLATED LEAVES.—The etiolated leaves used in these experiments were grown in the dark from mother beet roots. These leaves differed considerably from the green leaves produced in the light. Their petioles were flattened and elongated, and the lamina were poorly developed with disproportionately large veins. The vascular bundles, however, appeared normal.

Sugar gradients and percentages found in etiolated leaves are shown in table III. As in the young leaves, fructose was low. Equal quantities of fructose and glucose should have existed after the hydrolysis of sucrose, yet the concentration of fructose was always less than that of glucose. As in the

TABLE III

PERCENTAGES OF SUGARS IN ETIOLATED LEAVES FROM MOTHER BEET PLANTS GROWN IN THE DARKROOM. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
January 29			
Blade	0.55	0.05	0.14
Upper-petiole	1.85	0.60	0.07
Middle-petiole	1.96	0.65	0.11
Lower-petiole	1.95	0.68	0.30
February 9			
Blade	0.80	0.27	0.34
Upper-petiole	1.80	0.50	0.16
Middle-petiole	1.70	0.80	0.18
Lower-petiole	2.08	0.40	0.28

TABLE IV

COMPOSITION OF YOUNG LEAVES PRODUCED ON YOUNG PLANTS IN THE DARK. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PLANT PART	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
Blade	0.17	0.05	0.00
Petiole	1.07	0.43	0.00
Root	0.12	0.00	1.95

young leaves, some of the fructose was probably transformed into glucose or used in growth.

Sugar moved into the blades from the petioles. Evidently polarity differs in etiolated leaves and mature green leaves. Perhaps this difference in polarity is related to poor development of the mesophyll of etiolated leaves, or possibly to the immaturity and lack of differentiation in the etiolated leaves.

It is interesting to note that sucrose increased in concentration in passing from the petiole into the blade, thus bringing about a gradient reversal. This gradient was probably dependent, however, on the activity of the invertase and the sucrose-synthesizing systems in different parts of the leaf rather than on translocation.

Table IV shows the composition of young leaves produced in the dark on three-months-old beet plants. The sucrose content of the roots, at this stage, had been reduced to a low level for that tissue. Although sugars were moving into the young leaves from the roots, no sucrose was found in petioles or blades. Sucrose moving into the young leaves evidently was completely inverted. Perhaps this complete inversion was possible because the sugars moved into the leaves slowly as a result of low sugar level in the roots. The sugar content of the blade tissue was approximately 15 per cent. of that of the petiole and 10 per cent. of that of the root.

Transformation of sugars

Cut surfaces of petioles were dipped into sugar solutions. The blades were left exposed to the air to permit transpiration and draw the sugar solutions up the xylem into the leaves. Table V shows that the added sugars

TABLE V

Composition of blades and midribs before and after being fed sugar in feeding experiments

	Glucose		Fructose		Sucrose		Dextrin	
	Per cent.	Increase	Per cent.	Increase	Per cent.	Increase	Per cent.	Increase
	%	%	%	%	%	%	%	%
Initial composition								
Blades	0.17	.....	0.08	.....	0.20	.....	0.18	.....
Midribs	1.03	.....	0.00	.....	0.30	.....	0.26	.....
Fructose (3%)								
Blades	0.23	0.06	0.25	0.17	0.29	0.09	0.32	0.14
Midribs	1.63	0.60	0.39	0.39	0.69	0.39	0.38	0.12
Glucose (3%)								
Blades	0.30	0.13	0.22	0.14	0.35	0.15	0.34	0.16
Midribs	1.14	0.11	0.10	0.10	0.46	0.16	0.34	0.08
Sucrose (3%)								
Blades	0.55	0.38	0.40	0.32	0.31	0.11	0.49	0.31
Midribs	1.40	0.37	0.35	0.35	0.56	0.26	0.39	0.13

reached the leaf and that fructose was converted into glucose and glucose into fructose. The conversion of fructose into glucose was the more complete reaction, however. The dextrin-synthesizing mechanism was active. The sucrose response was not great, but here too there was synthesis.

Sucrose was hydrolyzed to the equilibrium point existing between the tissues as rapidly as it was absorbed and sucrose concentration was about the same whether the leaves were fed with the invert sugars or with sucrose.

Invertase was found to be very active in sugar beet blades and probably was responsible for the strong hydrolysis of the sucrose. Leaves fed either glucose or fructose synthesized some sucrose, but the synthesis was not strong. NURMIA (15) showed that leaves free or almost free of invertase were able to synthesize sucrose strongly. Evidently invertase is associated with sucrose inversion and not with sucrose synthesis. More evidence relating to this subject will be reported in another paper.

Discussion

Sugars migrated in detached mature leaves from the blades into the petioles, whether the leaves were placed in the sun or in the dark. This result is interpreted as indicating that sugars move from the blade mesophyll into the phloem in a polar direction. No suggestion is offered as to the mechanism involved. It is obvious that an expenditure of energy is necessary to move sugars from a region of low sugar content in the blades to a region of high concentration in the petioles. The polarizing force is strong, since blades were often found to contain no detectable sugar, even though sugars were present in the petiole in considerable percentages.

As long as there was a "sink" in the root of the sugar beets, the sugars traveled in that direction from the fully grown leaves. The use of carbohydrates seems to be of importance in determining the direction of translocation. Young leaves imported sugars readily, probably because the machinery of polarized movement had not become established. The leaves from young or rapidly growing sugar beet plants were always low in fructose, while the leaves from older or mature plants were always high in fructose. This condition could have been brought about entirely by factors affecting the glucose-fructose equilibrium. On the other hand, it is possible that fructose was used preferentially in growth. Since cellulose and other structural materials are considered to be glucosans, fructose may have been more readily transformed into "active" glucose than was glucose itself.

It is apparent that the relative proportions of the various carbohydrates in plant tissues are governed by a variety of transforming systems, as well as by other factors. Some of the factors which might be involved in determining the relative amounts of different carbohydrates within tissues are as follows: (1) The activities of the various carbohydrate-transforming sys-

tems; (2) the distribution of the carbohydrates within the cell; (3) the distribution of the carbohydrate-transforming systems within the cell; (4) the time available for the reactions; (5) the temperature; (6) growth and respiration; and (7) translocation.

The feeding experiments indicated that the various sugars were readily transformed into other forms. Leaves fed sucrose contained no more sucrose than those fed either glucose or fructose. If sucrose were formed by photosynthesis in the leaf instead of introduced artificially, we would expect the same degree of hydrolysis. In the same manner, if one of the reducing sugars was the first sugar of photosynthesis, we would expect it to be polymerized to the normal equilibrium point. Sugar transformations within the tissue prevent the use of diurnal accumulations as evidence that particular forms are produced in photosynthesis or are moved in a given transport system.

Summary

1. The sugar beet was used in studies on photosynthesis, translocation, and transformation of the sugars. It was found that fructose increased in the blades as the season progressed. A low level of fructose was associated with rapid growth and a higher level with slow growth and maturity of the plant.

2. Fructose, glucose, and sucrose all showed marked diurnal variations within the blades. Variations in fructose and glucose together were usually twice those of sucrose. Dextrin at times showed marked diurnal variations within the blades and is an important secondary product of photosynthesis.

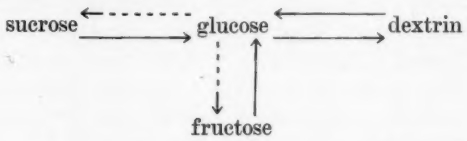
3. The sugars in mature leaves placed in the dark, whether the leaves were detached or on growing plants, continued to move out of the blades into the petioles until the blades were depleted of sugars. Evidently sugars move out of the blade mesophyll into the veins in a polar direction. There was, however, no tendency for any of the sugars from detached leaves to accumulate at the base of the petiole. Polarization thus appears to be a property of the border parenchyma of the blades.

4. Sugars of detached leaves placed in the sun moved out of the blade mesophyll and accumulated more or less evenly throughout the midribs and petioles. Again there was no tendency for any of the sugars to accumulate more in one section of the petiole than in another.

5. While there was a polarity which prevented the sugars from the phloem moving back into the blade mesophyll, no such polarity existed between the phloem and parenchyma of the petioles. During the daylight hours sugar passed out of the phloem and accumulated in the surrounding cells, while at night the reverse movement took place.

6. Etiolated leaves of beet plants contained considerably more glucose than fructose. Practically all of the sugar present in these leaves arose from sucrose.

7. In feeding experiments, fructose was converted into glucose and glucose into dextrin. There was some conversion of glucose into fructose. The diagram below, in a general way, illustrates the observed reactions. The broken lines indicate the weaker reactions.



The writer wishes to express his grateful thanks to Dr. W. E. Loomis, of Iowa State College, for his many helpful suggestions throughout the course of this investigation, and aid in preparation of the manuscript.

Mississippi Agricultural Experiment Station
State College, Mississippi

LITERATURE CITED

1. Barr, G. C. The polysaccharides of the vegetative tissues of maize. Unpublished thesis, Library, Iowa State College. 1935.
2. Bennett, C. W. Plant-tissue relations of the sugar-beet curly-top virus. Jour. Agr. Res. **48**: 665–701. 1934.
3. Bulgakova, Z., Giubbenet, E., and Liubimenko, V. On the mechanism of translocation of carbohydrates in the sugar beet. (Trans. title.) Physiol. Untersuch. Zuckerrübe. Erste Artikelserie. Ukrainisches Inst. Angew. Bot. Sect. Pflanzenphysiol. Charkiw. **1**: 213–233. 1930. (Cited from Biol. Abstr. **8**: 5449. 1934.)
4. Campbell, A. V. Carbohydrates of the mangold leaf. Jour. Agr. Sci. **4**: 248–259. 1912.
5. Clements, H. F. Mannose and the first sugar of photosynthesis. Plant Physiol. **7**: 547–550. 1932.
6. Davis, W. A., Daish, A. J., and Sawyer, G. C. Studies of the formation and translocation of carbohydrates in plants. I. The carbohydrates of the mangold leaf. Jour. Agr. Sci. **7**: 255–326. 1916.
7. Doby, G., and Hibbard, R. P. Nutrient ions of plants and the ion activation of plant enzymes. Jour. Biol. Chem. **73**: 405–416. 1927.
8. Engard, Charles. Pullman, Washington. Private communication. 1936.
9. Girard, M. A. Recherches sur la saccharogenie dans la betterave. Compt. Rend. Acad. Sci. **97**: 1305–1308. 1883; **99**: 808–811. 1884.
10. Leonard, O. A. Seasonal study of tissue function and organic solute movement in the sunflower. Plant Physiol. **11**: 25–61. 1936.
11. Lindet, M. L. Présence du dextrose et du levulose dans les feuilles de

betteraves. Ann. Agron. **26**: 103–113, 1900. (Cited from DAVIS, W. A. Jour. Agr. Sci. **7**: 327. 1916.)

12. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill. 1937.
13. MACQUENNE, M. L. Sur l'accumulation du sucre dans les racines de betterave. Compt. Rend. Acad. Sci. (Paris) **121**: 834–837. 1895.
14. MASON, T. G., and MASKELL, E. J. Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. Ann. Bot. **42**: 189–253. 1928.
15. NURMIA, M. Transformation of sugars in plants. Ann. Acad. Scientiarum Fennicae. ser. A. **44**: 1–105. 1935.
16. PEKLO, JAROSLAV. Histochemisches über die Lokalisation der Saccharose in der Zuckerrübe. Oest. Ung. Zeit. Zuckerind. **37**: 153. 1908. (Cited from DAVIS, DAISH, and SAWYER, p. 260.)
17. PHILLIS, E., and MASON, T. G. Studies on the transport of carbohydrates in the cotton plant. III. The polar distribution of sugar in the foliage leaf. Ann. Bot. **47**: 585–634. 1933.
18. ROBERTSON, R. A., IRVINE, J. C., and DOBSON, MILDRED E. A polarimetric study of the sucroclastic enzymes in *Beta vulgaris*. Biochem. Jour. **4**: 258–273. 1909.
19. RUHLAND, W. Untersuchungen über den Kohlenhydratstoffwechsel von *Beta vulgaris* (Zuckerrübe). Jahrb. wiss. Bot. **50**: 200–257. 1911.
20. STEPHANI, W. Untersuchungen über reduzierenden und nicht reduzierenden Zucker in den Beta-Rüben während des Wachstums und der Lagerung. Kühn-Archiv. **1**: 107–182. 1911.
21. STRAKOSCH, S. Ein Beitrag zur Kenntnis des Kohlenhydratstoffwechsels von *Beta vulgaris* (Zuckerrübe). Sitzber. Kais. Akad. Wiss. Wien. **116**: 855–869. 1907.
22. TOTTINGHAM, W. E., LEPKOVSKY, S., SCHULZ, E. R., and LINK, K. P. Climatic effects in the metabolism of the sugar beet. Jour. Agr. Res. **33**: 59–76. 1926.

FOLIAR DIAGNOSIS: THE INFLUENCE OF THE SOIL ON THE ACTION OF FERTILIZERS[1]

WALTER THOMAS AND WARREN B. MACK

(WITH TWELVE FIGURES)

Introduction

In an earlier paper (**6**) reference was made to the fact that no piece of land, however carefully selected for experimental purposes, is absolutely uniform. If the area is divided into equal sections and the sections sub-divided into plots of the same size, the yields from similarly treated duplicate plots planted on the same day with selected homogeneous seed will rarely be the same, and not infrequently will be widely divergent. For example, the relative percentage differences in yields—*i.e.* the value of $\left(\frac{x-y}{x}\times 100\right)$ where x and y are the respective yields of one pair of similarly treated plots—may differ by less than 5 per cent., whereas that of another pair may differ by 50 per cent. or more.

The relationship of the foliar diagnosis to the yields of potato plants growing on differently fertilized plots of tiers 1 and 2 of the vegetable fertility plots of the Pennsylvania Agricultural Experiment Station have been described in three earlier papers (**6, 7, 8**), familiarity with which is assumed in the present report. It was shown (**6, 8**) that the foliar diagnosis of a particular plot treatment was a characteristic of that plot and is related to the nature of the fertilizer applied and also to the development and yield of the plants growing thereon.

The object of the present investigation is to apply the method of foliar diagnosis (**6**) to an examination of the nature of the influence of the soil on the action of fertilizers, with special reference to plants of the same species growing on (1) a homogeneous soil and (2) a heterogeneous soil.

It has been repeatedly shown, (**1, 2, 8**), that when soils of two similarly treated plots are relatively homogeneous, as deduced from similar development and yields of the plants growing thereon, duplicate pairs will, in general, and with a sufficient degree of approximation for field experiments, be represented by similar foliar diagnosis. On the other hand, when the soils of two similarly treated plots are not uniform, as indicated by widely different development and yields of the plants growing thereon, they will be represented by dissimilar foliar diagnosis.

Consequently, markedly dissimilar foliar diagnoses of plants of the same species from plots treated alike is an indication of the heterogeneity of the

[1] Authorized for publication as paper no. 829 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

soils of the plots under comparison. These soil effects from duplicate plots may be the result not only of chemical differences but also of physical differences which may produce differences in the water or air supply to the roots.

Materials and methods

The present study was carried out on potato plants growing on plots from tiers, 1, 2, 4, and 5 of section B of the long-continued vegetable fertility experiments of the Pennsylvania Agricultural Experiment Station (**4**). The description of the experimental layout has been given by one of us (**4**). The analytical methods adopted were those described in an earlier paper (**6**).

Presentation of results

The treatment and yields of tubers from the duplicate plots are shown in table I. Tier 4 is the duplicate of tier 1, and tier 5 the duplicate of tier 2. The fourth column shows the relative differences in the yields of the respective duplicates in tiers 4 and 5 expressed as a percentage of the yield of the duplicate in tiers 1 and 2, respectively. The fifth and sixth columns show the mean *intensity of nutrition* and the mean *NPK-unit* of the fourth leaf (**6**). In the first column (A) designates the plots in tiers 1 and 2, and (B) those in tiers 4 and 5. The nitrogen, phosphoric acid, and potash contents of the fourth leaf from the base of the stalk at the various sampling dates, expressed (1) as percentage of each element in the dried foliage, (2) in milligram equivalents, and (3) as the composition of the *NPK-unit* are presented in tables II and III and are shown graphically in figures 1 to 12. Figure 1 indicates the percentages of N, P_2O_5, and K_2O in the dried foliage as ordinates, and dates of sampling as abscissae. In figures 2 to 10 the *NPK-units*—*i.e.* the (N–P_2O_5–K_2O) equilibria at the respective sampling dates—are plotted in trilinear coordinates, and the mean *NPK-unit* in figures 11 and 12.

Discussion and interpretation of results

The changes in the signs (+, –) in table I indicate that the differences in the yields of duplicate plots are not caused by a factor operating uniformly over the whole area. Thus, there are seven plots in tiers 1 and 2 in which the yield is greater (+) than that of the duplicate in tier 4 or 5, and six plots in which the yields in tiers 1 and 2 are less than (–) the respective duplicates in tier 4 or 5.

There are six plots in which the differences in the yields of duplicate plots are less than 10 per cent. and which may be regarded as falling within the degree of approximation that would occur in a relatively uniform soil. The relative percentage differences in yields $\left(\frac{x-y}{x} \times 100\right)$ of the remaining duplicate plots range from -12.9 to $+54.8$ per cent., showing that greater

TABLE I

RELATIVE PERCENTAGE DIFFERENCES IN YIELDS BETWEEN DUPLICATE PLOTS TOGETHER WITH THE MEAN INTENSITY OF NUTRITION AND THE MEAN NPK-UNIT

TREATMENT SYMBOL	TIER	PLOT	YIELDS	RELATIVE PERCENTAGE DIFFERENCE IN YIELDS	MEAN INTENSITY OF NUTRITION (TOTAL N + P_2O_5 + K_2O)	MEAN NPK-UNIT
			lb.	%		
2(NPK) (A)	2	16	170	+ 4.7	11.45	63.61 : 4.78 : 31.60
2(NPK) (B)	5	2	162		10.66	65.63 : 4.72 : 29.65
0.5(NPK) (A)	2	14	150	- 5.3	8.79	68.85 : 5.44 : 25.70
0.5(NPK) (B)	5	4	158		8.93	66.05 : 5.36 : 28.59
(1.5N)PK (A)	2	4	157	+ 5.7	9.74	69.15 : 4.80 : 26.05
(1.5N)PK (B)	5	14	148		10.39	66.59 : 4.27 : 29.14
NK (A)	1	7	163	- 6.7	10.22	66.96 : 4.41 : 28.63
NK (B)	4	11	174		10.91	65.47 : 3.94 : 30.59
Manure (A)	1	15	196	+ 7.2	10.33	65.0 : 5.72 : 29.28
Manure (B)	4	3	182		10.29	65.95 : 5.68 : 28.37
NP(1.5K) (A)	2	12	174	+ 9.8	10.74	63.68 : 4.94 : 31.38
NP(1.5K) (B)	5	6	157		9.57	65.42 : 5.05 : 29.54
NP (A)	1	6	124	- 12.9	6.10	84.18 : 6.25 : 9.56
NP (B)	4	12	140		7.40	78.94 : 5.67 : 15.39
N (A)	1	2	109	+ 19.2	7.34	81.12 : 4.91 : 13.97
N (B)	4	16	88		7.52	77.43 : 5.06 : 17.50
N(1.5P)K (A)	2	8	176	- 19.4	9.62	65.94 : 5.47 : 28.58
N(1.5P)K (B)	5	10	210		9.70	66.03 : 5.56 : 28.40
NPK (A)	1	10	162	- 20.3	9.39	68.65 : 5.15 : 26.19
NPK (B)	4	8	195		10.03	67.44 : 4.97 : 27.58
P (A)	1	3	114	+ 22.8	5.65	81.76 : 7.29 : 10.95
P (B)	4	15	88		7.28	70.95 : 6.69 : 22.37
PK (A)	1	8	148	- 40.5	9.58	63.29 : 5.64 : 31.06
PK (B)	4	10	208		9.50	63.90 : 5.62 : 30.33
K (A)	1	4	155	+ 54.8	9.48	65.91 : 4.88 : 29.20
K (B)	4	14	70		9.32	63.37 : 4.66 : 31.97

TABLE II

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P_2O_5 AND K_2O IN DRY FOLIAGE AND THE SUM $N + P_2O_5 + K_2O$ TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS

TIERS 1 AND 2

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS				COMPOSITION OF THE NPK-UNIT		
			N	P_2O_5	K_2O	$M_x + M_y + M_z$	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
			M_x	M_y	M_z	s	E_x	E_y	E_z	S	$\left(100\frac{E_x}{S}\right)$	$\left(100\frac{E_y}{S}\right)$	$\left(100\frac{E_z}{S}\right)$
			%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
July 7	2(NPK)	2 No. 16	5.23	0.682	8.771	14.683	373.422	28.849	186.822	589.093	63.389	4.897	31.713
July 29			4.04	0.562	5.930	10.532	288.456	23.773	126.309	438.538	65.777	5.420	28.802
August 9			3.87	0.500	6.124	10.494	276.318	21.150	130.441	427.909	64.574	4.943	30.483
August 24			3.50	0.354	6.453	10.107	235.620	14.974	137.449	388.043	60.720	3.858	35.422
July 7	0.5(NPK)	2 No. 14	4.84	0.520	6.108	11.468	345.576	21.996	130.100	497.672	69.439	4.418	26.142
July 29			3.77	0.528	4.379	8.677	269.178	22.334	93.273	384.785	69.957	5.803	24.239
August 9			3.34	0.466	4.031	7.837	238.476	19.712	85.860	344.048	69.315	5.729	24.956
August 24			2.84	0.418	3.920	7.178	202.776	17.681	83.496	303.953	66.715	5.817	27.468
July 7	(1.5 N)PK	2 No. 4	5.23	0.614	6.780	12.624	373.422	25.972	144.414	543.808	68.667	4.775	24.555
July 29			4.00	0.488	5.040	9.528	285.600	20.642	107.352	413.594	69.054	4.990	24.956
August 9			3.98	0.464	4.534	8.978	284.172	19.627	96.574	400.373	70.977	4.903	24.120
August 24			3.16	0.356	4.302	7.818	225.624	15.059	91.633	332.316	67.892	4.532	27.572
July 7	NK	1 No. 7	5.10	0.464	6.630	12.194	364.140	19.627	141.176	524.943	69.367	3.739	26.894
July 29			4.03	0.510	5.550	10.090	287.742	21.573	118.300	427.615	67.290	5.045	27.665
August 9			3.78	0.452	5.280	9.512	269.892	19.119	112.443	401.454	67.229	4.763	28.008
August 24			3.26	0.352	5.460	9.072	232.764	14.889	116.234	363.887	63.964	4.092	31.941
July 7	Manure	1 No. 15	4.65	0.688	7.550	12.888	332.010	29.102	160.815	521.927	63.612	5.575	30.812
July 29			3.80	0.608	5.128	9.536	271.320	25.718	109.226	406.264	66.785	6.331	26.887
August 9			3.79	0.564	5.155	9.509	270.606	23.857	109.801	404.264	66.939	5.902	27.161
August 24			3.28	0.448	5.658	9.386	234.192	18.950	120.515	373.657	62.675	5.071	32.254
July 7	NP(1.5K)	2 No. 12	4.86	0.594	7.798	13.252	347.004	25.126	166.097	538.227	64.470	4.669	30.860
July 29			4.19	0.522	5.767	10.479	299.166	22.081	122.837	444.084	67.368	4.972	24.662
August 9			3.71	0.513	5.782	10.005	264.894	21.699	123.157	409.750	64.647	5.296	30.057
August 24			2.82	0.394	5.996	9.210	201.348	16.666	127.715	345.729	58.239	4.822	36.939
July 7	NP	1 No. 6	5.14	0.590	2.530	8.260	366.996	24.957	53.910	445.863	82.311	5.598	12.091
July 29			3.87	0.532	1.520	5.922	276.318	22.504	32.355	331.177	83.435	6.794	9.771
August 9			3.71	0.486	1.190	5.386	264.894	20.558	25.262	310.714	85.253	6.617	8.130
August 24			3.36	0.390	1.090	4.840	239.904	16.751	23.110	279.765	85.752	5.987	8.261

August 24			3.36	0.390	1.090	4.840	239.904	16.751	23.110	279.765	85.752	5.987	8.261

TABLE II—(*Continued*)

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P_2O_5 AND K_2O IN DRY FOLIAGE AND THE SUM $N + P_2O_5 + K_2O$ TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS

TIERS 1 AND 2

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS				COMPOSITION OF THE NPK-UNIT		
			N	P_2O_5	K_2O	$M_x + M_y + M_z$	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
			M_x	M_y	M_z	S	E_x	E_y	E_z	S	$\left(100\frac{E_x}{S}\right)$	$\left(100\frac{E_y}{S}\right)$	$\left(100\frac{E_z}{S}\right)$
			%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
July 7	N	1 No. 2	5.12	0.404	3.970	9.494	365.568	17.089	84.561	467.218	78.244	3.658	18.098
July 29			4.42	0.534	2.540	7.494	315.588	22.588	54.102	392.278	80.450	5.759	13.791
August 9			4.15	0.472	2.010	6.632	296.310	19.966	42.813	359.089	82.517	5.561	11.922
August 24			3.64	0.344	1.770	5.754	259.896	14.551	37.701	312.148	83.261	4.661	12.078
July 7	N(1.5P)K	2 No. 8	4.84	0.710	6.969	12.519	345.576	30.033	148.440	524.049	65.944	5.730	28.325
July 29			3.72	0.564	4.914	9.198	256.608	23.857	104.668	394.133	67.391	6.054	26.557
August 9			3.46	0.482	4.934	8.876	247.044	20.389	105.094	372.527	66.314	5.473	28.209
August 24			2.86	0.350	4.670	7.880	204.204	14.805	99.471	318.480	64.117	4.650	31.233
July 7	NPK	1 No. 10	4.98	0.584	6.589	12.153	355.570	24.70	140.34	520.62	68.30	4.74	26.96
July 29			3.88	0.509	4.337	8.726	277.03	21.53	92.38	390.94	70.86	5.51	23.63
August 9			3.62	0.482	4.697	8.799	258.47	20.39	100.05	376.90	68.22	5.38	26.40
August 24			3.14	0.392	4.348	7.880	224.19	16.58	92.61	333.39	67.25	4.97	27.78
July 7	P	1 No. 3	4.57	0.550	2.41	7.530	326.298	25.042	51.333	402.673	81.034	6.218	12.747
July 29			3.46	0.521	1.64	5.621	247.044	22.038	34.932	304.014	81.260	7.250	11.490
August 9			3.24	0.520	1.29	5.050	231.336	21.996	27.477	280.809	82.383	7.831	9.786
August 24			2.79	0.450	1.11	4.350	199.206	19.035	26.643	241.884	82.359	7.868	9.773
July 7	PK	1 No. 8	4.55	0.620	6.76	11.930	324.870	26.395	143.967	495.232	65.599	5.330	29.071
July 29			3.36	0.510	5.32	9.190	239.904	21.573	113.337	374.814	64.006	5.755	30.239
August 9			3.08	0.490	5.35	8.920	219.912	20.980	113.912	354.804	61.981	5.913	32.105
August 24			2.82	0.430	5.04	8.290	201.348	18.189	107.309	326.846	61.603	5.565	32.832
July 7	K	1 No. 4	4.61	0.424	6.32	11.354	329.154	17.935	134.573	481.662	68.336	3.725	27.939
July 29			3.87	0.540	5.22	9.630	276.318	22.842	111.122	410.282	67.349	5.567	27.084
August 9			3.39	0.464	4.91	8.764	242.046	19.627	104.689	366.362	66.068	5.358	28.575
August 24			2.78	0.370	5.00	8.150	198.492	15.651	106.500	320.643	61.904	4.881	33.215

TABLE III

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P_2O_5 AND K_2O IN DRY FOLIAGE AND THE SUM $N + P_2O_5 + K_2O$ TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS

DUPLICATE PLOTS TIERS 4 AND 5

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS				COMPOSITION OF THE NPK-UNIT		
			N	P_2O_5	K_2O	$M_x + M_y + M_z$	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
			M_x	M_y	M_z	s	E_x	E_y	E_z	S	$100\frac{E_x}{S}$	$100\frac{E_y}{S}$	$100\frac{E_z}{S}$
			%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
July 7	2(NPK)	5 No. 2	5.03	0.650	7.521	13.201	359.142	27.495	160.197	546.834	65.676	5.028	29.292
July 29			4.08	0.509	5.542	10.131	291.312	21.531	118.045	430.888	67.607	4.997	27.396
August 9			3.76	0.454	5.658	9.872	268.464	19.204	120.515	408.183	65.770	4.705	29.525
August 24			3.35	0.368	5.775	9.493	239.190	15.566	123.007	377.763	63.317	4.120	32.562
July 7	0.5(NPK)	5 No. 4	4.34	0.524	6.705	11.569	309.876	22.165	142.816	474.857	65.257	4.668	30.075
July 29			3.57	0.486	4.883	8.939	254.898	20.558	104.008	379.464	67.173	5.418	27.409
August 9			3.12	0.454	4.310	7.884	222.768	19.204	91.803	333.775	66.742	5.753	27.504
August 24			2.75	0.400	4.162	7.312	196.350	16.920	88.651	301.921	65.033	5.604	29.362
July 7	(1.5N)PK	5 No. 14	4.98	0.528	6.814	12.322	355.572	22.334	145.138	523.044	67.981	4.270	27.748
July 29			4.26	0.446	5.744	10.450	304.164	18.866	122.347	445.377	68.293	4.235	27.470
August 9			3.76	0.430	5.705	9.895	268.464	18.189	121.516	408.169	65.772	4.456	29.771
August 24			3.23	0.348	5.310	8.888	230.622	14.720	113.103	358.445	64.339	4.106	31.553
July 7	NK	4 No. 11	5.04	0.412	6.790	12.242	359.856	17.428	144.627	521.911	68.949	3.339	27.711
July 29			4.31	0.498	5.968	10.776	307.734	21.065	127.118	455.917	67.498	4.620	27.882
August 9			3.78	0.420	6.269	10.469	269.892	17.766	133.530	421.188	64.079	4.218	31.703
August 24			3.36	0.332	6.434	10.126	239.904	14.044	137.044	390.992	61.358	3.592	35.050
July 7	Manure	4 No. 3	4.84	0.750	7.248	12.838	345.576	31.725	154.382	531.683	64.997	5.967	29.036
July 29			4.04	0.604	5.116	9.760	288.456	25.549	108.971	422.976	68.197	6.040	25.763
August 9			3.58	0.502	4.988	9.070	255.612	21.235	106.244	383.091	66.723	5.543	27.733
August 24			3.45	0.472	5.601	9.523	246.330	19.966	119.301	385.597	63.883	5.178	30.939
July 7	NP(1.5K)	5 No. 6	4.54	0.554	6.794	11.888	324.156	23.434	144.712	492.302	65.845	4.760	29.395
July 29			3.71	0.502	5.124	9.336	264.894	21.235	109.141	395.270	67.015	5.372	27.612
August 9			3.26	0.444	4.653	8.357	232.764	18.781	99.109	350.654	66.379	5.356	28.272
August 24			3.01	0.382	5.310	8.702	214.914	16.159	113.103	344.176	62.443	4.695	32.862
July 7	NP	4 No. 12	5.08	0.558	4.632	10.270	362.712	23.603	98.662	484.977	74.789	4.867	20.344
July 29			4.20	0.526	2.635	7.361	299.880	22.249	56.125	378.254	79.280	5.882	14.838
August 9			3.78	0.480	2.170	6.430	269.892	20.304	46.221	336.417	80.225	6.035	13.739
August 24			3.37	0.412	1.755	5.537	240.618	17.428	37.381	295.427	81.447	5.899	12.653

August 9			3.78	0.480	2.170	6.430	269.892	20.304	40.221	330.417	81.667	5.899	12.434
August 24			3.37	0.412	1.755	5.537	240.618	17.428	37.381	295.427	81.447	5.899	12.653

TABLE III—(*Continued*)

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P_2O_5 AND K_2O IN DRY FOLIAGE AND THE SUM $N + P_2O_5 + K_2O$ TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS

DUPLICATE PLOTS TIERS 4 AND 5

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE				MILLIGRAM EQUIVALENTS				COMPOSITION OF THE NPK-UNIT		
			N	P_2O_5	K_2O	$M_x + M_y + M_z$	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
			M_x	M_y	M_z	s	E_x	E_y	E_z	S	$100\frac{E_x}{S}$	$100\frac{E_z}{S}$	$100\frac{E_z}{S}$
			%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
July 7	N	4 No. 16	4.71	0.424	4.438	9.572	336.294	17.935	94.529	448.758	74.939	3.996	21.064
July 29			4.11	0.502	3.139	7.751	293.454	21.235	66.861	381.550	76.911	5.565	17.523
August 9			3.75	0.458	2.635	6.843	267.750	19.373	56.125	343.348	78.005	5.644	16.351
August 24			3.40	0.362	2.151	5.913	242.760	15.313	45.816	303.889	79.884	5.039	15.076
July 7	N(1.5P)K	5 No. 10	4.84	0.658	6.678	12.176	345.576	27.833	142.241	515.650	67.017	5.397	27.584
July 29			3.90	0.608	5.031	9.539	278.460	25.718	107.160	411.338	67.696	6.252	26.051
August 9			3.40	0.476	4.883	8.759	242.760	20.135	104.008	366.903	66.164	5.488	28.348
August 24			2.96	0.404	4.961	8.325	211.344	17.089	105.669	334.102	63.257	5.115	31.628
July 7	NPK	4 No. 8	4.92	0.604	6.907	12.431	351.288	25.549	147.119	523.956	67.043	4.876	28.078
July 29			4.36	0.542	5.129	10.031	311.304	22.927	109.248	443.479	70.196	5.169	24.634
August 9			3.62	0.460	5.000	9.080	258.468	19.458	106.500	384.426	67.209	5.061	27.704
August 24			3.23	0.398	4.961	8.589	230.622	16.835	105.669	353.126	65.309	4.767	29.924
July 7	P	4 No. 15	4.30	0.548	5.155	10.003	307.020	23.180	109.695	439.895	69.794	5.269	24.937
July 29			3.38	0.512	3.488	7.380	241.332	21.658	74.294	337.284	71.551	6.421	22.027
August 9			3.06	0.514	3.042	6.616	218.484	21.742	64.752	304.978	71.639	7.129	21.232
August 24			2.34	0.444	2.352	5.136	167.076	18.781	50.055	235.912	70.821	7.961	21.218
July 7	PK	4 No. 10	4.45	0.636	6.651	11.737	317.730	26.903	141.666	486.299	65.336	5.532	29.131
July 29			3.64	0.580	5.193	9.413	259.896	24.534	110.611	395.041	65.789	6.210	27.999
August 9			3.10	0.484	4.872	8.456	221.340	20.473	103.773	345.586	64.048	5.924	30.028
August 24			2.75	0.370	5.300	8.420	196.350	15.651	112.890	324.891	60.436	4.817	34.131
July 7	K	4 No. 14	4.10	0.432	6.317	10.849	292.740	18.274	134.552	445.566	65.701	4.101	30.198
July 29			3.50	0.452	5.232	9.184	249.900	19.119	111.442	380.461	65.693	5.025	29.291
August 9			3.20	0.432	5.496	9.128	228.480	18.274	117.065	363.819	62.800	5.023	32.177
August 24			2.56	0.328	5.242	8.130	182.784	13.874	111.655	308.313	59.285	4.499	36.215

differences exist in the soils of these like-treated pairs of plots than in the other groups of six plots.

The highest difference between the yields of duplicate plots is that between the K plots (no. 1–4 and no. 4–14). This is associated with a peculiar phenomenon which accompanies the absorption of potassium (*i.e.*, potash nutrition), which will be discussed in later papers.

Graphic representation of percentages of N, P_2O_5 and K_2O in the dried foliage as ordinates and dates of sampling as abscissae

The method of interpreting such graphs has been given in earlier papers (**6, 7, 8**). An inspection of the graphs (fig. 1) shows that much greater dif-

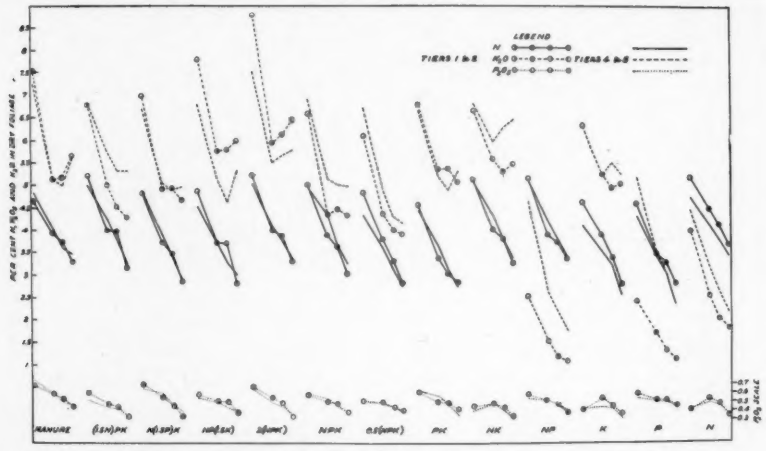


Fig. 1. The percentages of N, P_2O_5 and K_2O in the fourth leaf with advancing age.

ferences occur in the potash content of couplets, with advancing age of the leaf, than in the nitrogen or phosphoric acid contents. In two couplets not receiving any mineral potash applications (P and NP) the differences are phenomenal, showing the marked differences in the character of the soil of these couplets and its influence on the K nutrition of the plant.

The characteristics of the graphs in figure 1 which appear most frequently in connection with differences in yield of duplicate plots are the following:

Among the couplets with relatively high yields (average 150 pounds or more) which differed less than 10 per cent., the plants growing on the plot with greater yield are characterized by a more rapid utilization of K_2O at a higher level than those from the lower yielding plot, especially during the early part of the sampling period. Exceptions are (1.5N)PK, in which,

though utilization is greater throughout the season in the duplicate with highest yield, the level of K_2O is lower at the end of the sampling period; and NK, in which the plot with the lowest yield, (no. 1–7), has higher utilization at the beginning of the sampling period, but reaches a lower level at the second sampling date.

Among couplets with relatively low yields (average less than 150 pounds) or with high yields differing by more than 10 per cent., more rapid utilization of K_2O during part or all of the season at a higher level is more often associated with lower yield. An exception is NP, in which the more rapid utilization at the higher level characterizes the plot (no. 4–12) with the greater yield.

The form of the K_2O graphs is similar for duplicate plots of which the yields differ less than 10 per cent., with the exception of NK, in the respect that utilization is very rapid at the beginning, and is distinctly slower at the end of the sampling period; accumulation sometimes occurs at the latter time.

The form of K_2O graphs is dissimilar for duplicate plots of which the yields differ more than 10 per cent. from each other, except in NP, P, and N, in the first two of which the contents of K_2O in the leaf are markedly different in the duplicate plots.

No consistent relation between either N, P_2O_5, or the balance between N and P_2O_5 utilization (5) and the yield of duplicates may be observed.

Graphic representation of the NPK-units in trilinear coordinates

Course of nutrition with respect to nitrogen, phosphoric acid, and potash from one sampling date to another.—The method of deriving the *NPK-unit* and of interpreting the graphs has been given in an earlier paper (6). Displacement upwards toward the summit of the triangle (N = 100 per cent.) indicates an increase in the nitrogen of the *NPK-unit;* displacement to the left base apex (K_2O = 100 per cent.), an increase in the potash content of the *NPK-unit;* and displacement towards the right base apex (P_2O_5 = 100 per cent.), an increase in the P_2O_5 content of the *NPK-unit.* The solid lines (figs. 2–10) indicate the equilibrium between nitrogen, phosphoric acid, and potash at successive dates of sampling (indicated by the numerals 1, 2, 3, 4) of the fourth leaf of plants growing on tiers 1 and 2 and marked (A) in table I, and the broken lines indicate that of the respective duplicate plots in tiers 4 and 5, marked (B) in table I.

It should be kept in mind that a fertilizer may intervene to produce (a) a change in the *intensity of nutrition* or (b) a change in the N–P_2O_5–K_2O equilibrium (*NPK-unit*) or a change in (a) and (b) simultaneously (6).

The eye will be able to follow the differences in the N–P_2O_5–K_2O equilibrium between the duplicate pairs, with the advancing age of the leaf, without the necessity of any detailed description of these differences.

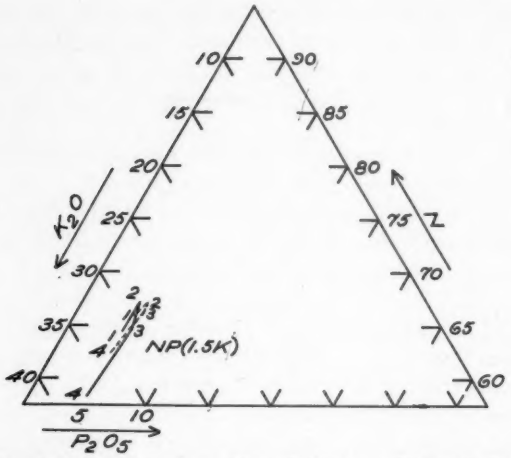


FIG. 2. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [NP(1.5K)].

The fact that the *NPK-unit* in no case ever follows the direction first taken is indicated by the zig-zag appearance of the graphs. The changes in the N–P_2O_5–K_2O equilibrium of a particular plot graph from one sampling date to another are the result of meteorological influences during growth and development, and the different forms of the graphs show that this factor does not act identically over all treatments.

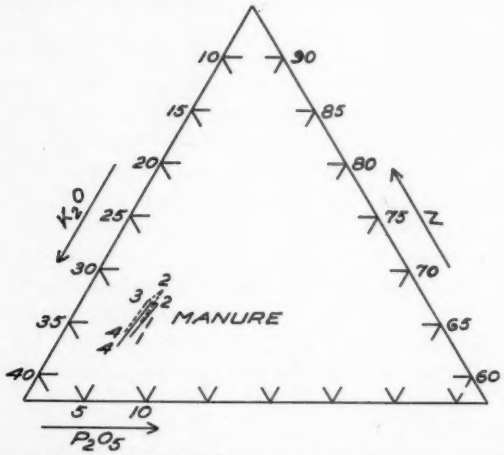


FIG. 3. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates (manure).

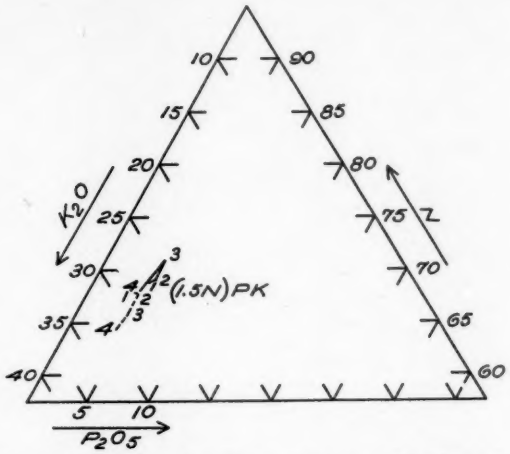


FIG. 4. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [(1.5N)PK].

From the manner in which the *NPK-units* are derived (**6**) one would expect that the relative changes in direction of an element in the *NPK-unit* would be identical with the changes in the direction of the graphs of that element expressed as a percentage of the dried foliage for the period considered (fig. 1). This is in general the case. The deviations from the rule are found in the nitrogen or phosphorus of the following couplets: NP(1.5K)

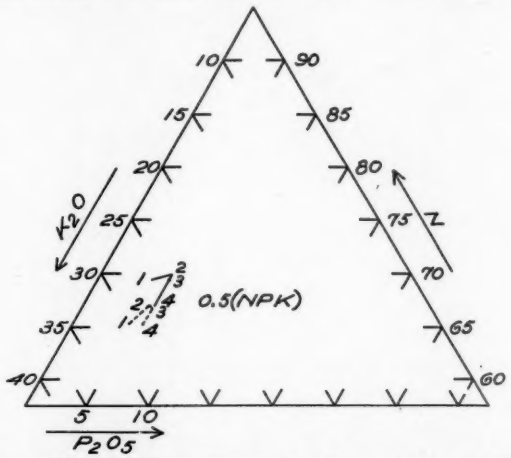


FIG. 5. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [0.5(NPK)].

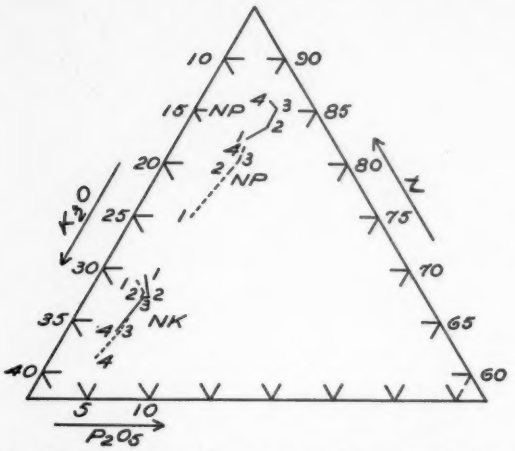


FIG. 6. The relative courses of nutrition of plants on duplicate NP and NK plots respectively; the NK plots differ from each other by less than 10 per cent. in yields, and the NP plots by more than 10 per cent.

(no. 2–12 and no. 5–6), NPK (no. 1–10 and no. 4–8) NK (no. 1–7 and no. 4–11), and NP (no. 2–12 and no. 5–6). These divergences are the result of the relatively large differences in the percentage of potash in the leaves of plants growing on the above duplicate plots.

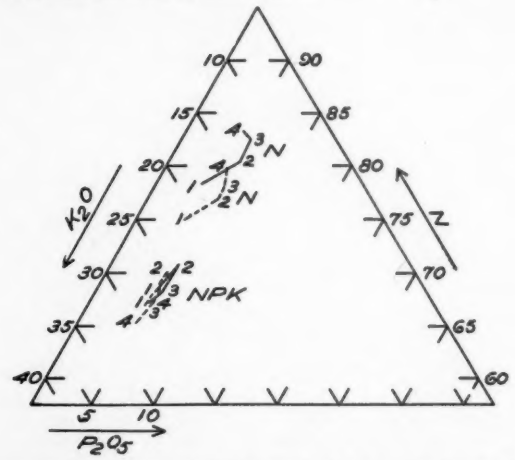


FIG. 7. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates.

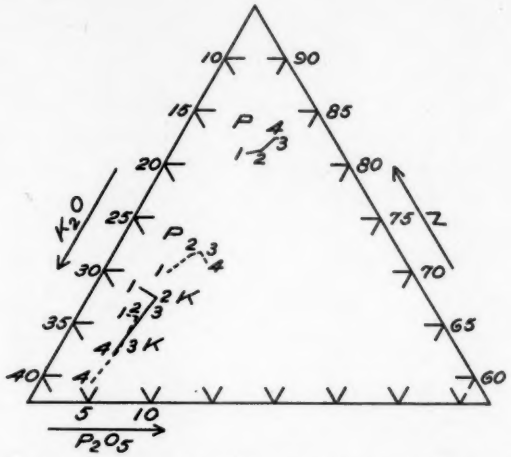


FIG. 8. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates.

The displacements from one sampling date to another (figs. 2–10) showing the course of nutrition, with respect to nitrogen, phosphoric acid, and potash are rarely identical in the duplicates. In some pairs of duplicates the divergences may be so great that the trilinear coordinate diagrams may be

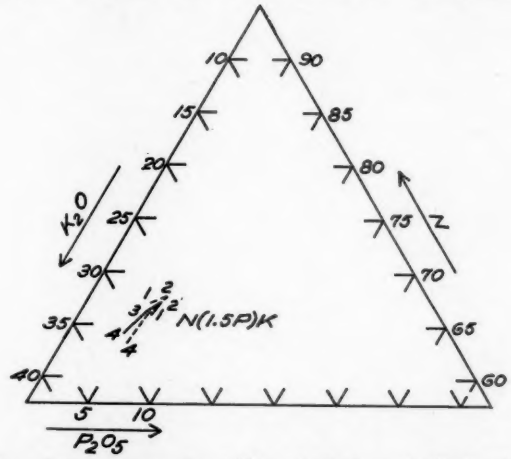


FIG. 9. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates [N(1.5P)K].

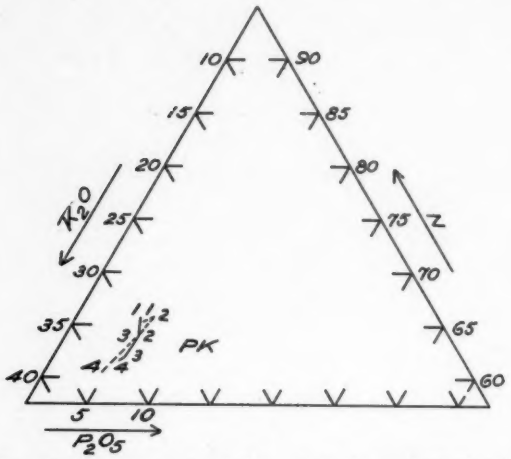


FIG. 10. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates [PK].

distinctly separated at all sampling dates [figs. 3, 5, 6 (NP), 7 (N), 8 (P)] and in others the diagrams may overlap and the lines of one member of a pair may intersect or nearly coincide with those of its duplicate [figs. 2, 4, 6 (NK), 7 (NPK), 8 (K), 9, 10]. In the latter, the detailed graphs are not sharply differentiated.

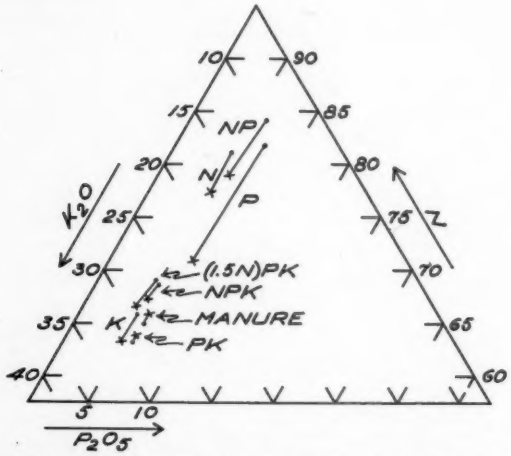


FIG. 11. The relative positions of the mean *NPK-unit* of duplicate plots. The position of the duplicate in tier 4 or tier 5 is indicated by a cross (×).

RELATION OF THE MEAN NPK-UNITS OF SIMILARLY TREATED COUPLETS.—The indications given by the trilinear coordinate diagrams can be analyzed more readily by an examination of figures 11 and 12 which show the mean *NPK-units* in such a way that the points representing the mean *NPK-units* of duplicate pairs of treatments are joined by a straight line. A cross (×) indicates the mean *NPK-unit* of the corresponding plot in tiers 4 and 5. The mean *NPK-unit* corresponds to the center of gravity of the respective diagrams in figures 2–10.

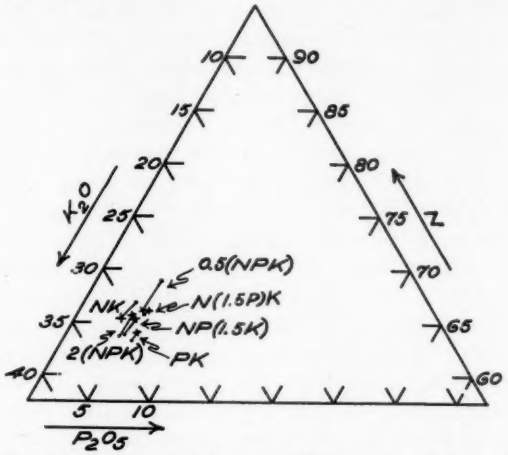


FIG. 12. The relative positions of the mean *NPK-units* of duplicate plots. The position of the duplicate in tier 4 or in tier 5 is shown by a cross (×).

The differences in the displacements of the mean *NPK-units* (figs. 11 and 12 and table I) between duplicate pairs are relatively less for certain heavily fertilized plots [manure, 2(NPK), N(1.5P)K, NP(1.5K)] than for plots with relatively lower fertilization (N, NP, P). The displacement of the duplicate mean *NPK-unit* toward the left base apex, indicating higher K_2O and lower N in the *NPK-unit*, occurs with higher yield in 2(NPK), 0.5(NPK), manure, NK, NP(1.5K), and NPK, all of which have yields above 150 pounds, and all differ less than 10 per cent. between duplicates; NP, of which the duplicates differ by 12.9 per cent., also has relatively higher K_2O in the mean *NPK-unit* of the higher-yielding plot. On the other hand, the displacement of the *NPK-unit* is toward the vertical apex (lower K_2O and higher N) for the duplicate with higher yield in N, P, K, PK, and (1.5N)PK, all except the last of which differ by more than 12.9 per cent. between the yields of duplicate plots. With respect to the relative composition of the mean *NPK-unit*, therefore, the yields of duplicate N, P, K, and PK plots are anomalous.

Displacements of duplicate *NPK-units* with respect to the right base apex show no consistent relations with relative yield.

Accompanying these differences in the N–P_2O_5–K_2O equilibrium (*i.e.*, the *NPK-unit*) between duplicates, the differences in the *intensities of nutrition* of the respective duplicates may be great, medium, or small. In the group of seven plots showing small relative differences in yields between duplicates [2(NPK, 0.5(NPK), (1.5N)PK, NK, manure, NP(1.5K) and NP] a higher *intensity of nutrition* is accompanied by higher yields, except in one case—*viz.*, (1.5N)PK (no. 2–4 and no. 5–14). In the group of six plots showing large differences in yields of duplicates [N, N(1.5P)K, NPK, P, PK, and K] a higher *intensity of nutrition* is accompanied by higher yields in three cases and by lower yields in the other three; the latter are N, P, and PK, which as indicated above are anomalous in other respects.

There are, accordingly, nine out of the thirteen pairs of duplicates in which a higher *intensity* is accompanied by higher yields. In seven of these nine cases, the higher *intensity* is the result of an increase in the potash of the *NPK-unit* made at the expense of the nitrogen. In the other two cases—*viz.*, K(no. 1–4 and no. 4–14) and N(1.5P)K (no. 2–8 and no. 5–10)—the higher *intensity* of the higher yielding duplicate is the result of higher nitrogen in the *NPK-unit* made at the expense of the phosphoric acid and potash.

The four pairs of duplicates in which higher *intensity of nutrition* is accompanied by lower yields have abnormal positions on the triangle. In one pair of duplicates—*viz.*, (1.5N)PK (no. 2–4 and no. 5–14)—the lowest yielding plot (no. 5–14) has a very low phosphoric acid content in the *NPK-unit* and lies further away from the apex of the triangle where $P_2O_5 = 100$ per cent. than in any of the plots except the NK plot (no. 4–11). Three pairs of duplicates—*viz.*, N(no. 1–2 and no. 4–16), P(no. 1–3 and no. 4–15), and NP(no. 1–6 and no. 4–12)—are situated much higher on the triangle towards the apex $N = 100$ per cent. than the other plots.

The relations of the foliar diagnosis of the N duplicates (no. 1–2 and no. 4–16) and also of the P duplicates (no. 1–3 and no. 4–15) are anomalous, as has been shown, both with respect to the *intensities* and the *NPK-units*. In these plots both the *intensity of nutrition* and the *NPK-units* of the higher yielding duplicate is further removed from the optimum *intensity* and the optimum *NPK-unit*.

The evidence derived from more recent work in the Jordan fertility plots of this Agricultural Experiment Station is that this abnormality is the result of differences in calcium, the effect of which is to reduce the potash and increase the nitrogen and phosphoric acid in the *NPK-unit*.

Because of the topography of plots no. 4–14 (K), no. 4–15 (P), and no. 4–16 (N), some of the subsurface clay is exposed by erosion. The pronounced acidic functions of clay apparently have caused factors other than the ones

varied (nitrogen, phosphoric acid, and potash) to produce the differences in yields.

As already pointed out (**6**), plot experiments are based on the assumption that all the other factors are equal except those which are varied. If the calcium or magnesium or any other factor is greatly different in two plots, the foliar diagnosis with respect to N, P_2O_5, and K_2O might be inadequate to explain the differences in yield. Calcium and magnesium could not be determined in these experiments because of the use of calcium-containing sprays. The foliar diagnosis of potato plants sprayed with non-calcium-containing sprays is now under investigation.

The PK duplicates (no. 1–8 and no. 4–10) differ by over 40 per cent. and yet the mean *intensities of nutrition* and the mean *NPK-units* differ but little. Differences that exist in nutrition, slight as they are, have been shown to be related to differences in yield in a manner inconsistent with that found in other plots. One must, therefore, assume also in the case of these two duplicates the existence of a factor or factors other than N, P_2O_5, or K_2O which is the cause of the differences in yield.

Considering all tiers, the highest yields have been obtained with *NPK-units* within limits of $N : P_2O_5 : K_2O = 63.9–67.4 : 5.0–5.7 : 27.7–30.3$ and *intensities* within limits of 9.5–10.3. Departure from either one or more of these limits has resulted in yields of less than 195 pounds per 1/100-acre plot.

Summary

1. The foliar diagnoses of similarly fertilized duplicate pairs of plots from a long-continued field experiment with potatoes were compared. The fertilizer treatments consisted of a single element, combinations of two elements, combinations of all three elements differently equilibrated, and also a manure treatment.

2. Soil heterogeneity is indicated by the relative yields of tubers from duplicate plots. The differences in yields between similarly treated couplets varied from 4.7 per cent. to 54.8 per cent. The divergences were not the result of a factor operating uniformly over the whole area.

3. The differences in the course of nutrition with respect to nitrogen, phosphorus, and potassium resulting from the several fertilizer treatments are shown graphically.

4. The magnitude of the displacements of the *NPK-units* ($N–P_2O_5–K_2O$ equilibrium) from one sampling date to another is never quite identical in the duplicates, although the directions of the displacements are similar.

5. The meteorological factors have not acted identically on all treatments.

6. The relative positions of the mean *NPK-unit* of couplets indicate that in six high-yielding couplets [manure, 2(NPK), N(1.5P)K, NP(1.5K), NK, and NP] differing in yield by 12 per cent. or less, the higher yielding duplicate

has higher K_2O and lower N in the *NPK-unit* and a higher *intensity of nutrition*. In four low-yielding duplicates having large differences in yields between duplicates (N, P, K, PK) the higher yielding duplicate has higher N and lower K_2O in the *NPK-unit* with lower *intensity of nutrition* (P) or with intensities nearly identical (N, K, PK).

7. The four couplets in which higher *intensity of nutrition* is accompanied by lower yields have abnormal positions on the triangle. The abnormalities are believed to be the result of differences in the available Ca and Mg of the duplicate plots. Work is under way to test this hypothesis.

THE LABORATORY OF PLANT NUTRITION
DEPARTMENT OF HORTICULTURE
THE PENNSYLVANIA STATE COLLEGE

LITERATURE CITED

1. LAGATU, H. and MAUME, L. Le diagnostic foliare de la pomme de terre. Ann. École Nat. Agr. Montpellier. Premier mémoire **20**: 219–281. 1930.
2. ————, and ————. Le diagnostic foliare de la pomme de terre. Ann. École Nat. Agr. Montpellier. Deuxième mémoire **22**: 50–126. 1932.
3. ————, and ————. Sur le contrôle chimique du mode d'alimentation de la vigne par les engrais. Commun. Con. Engrais Azotés de Synthèse à Montpellier. Juin 1. pp. 1–15. 1927.
4. MACK, WARREN B. Fertilization of truck crops in rotation. Pennsylvania Agr. Exp. Sta. Bull. 210. 1927.
5. THOMAS, WALTER. Mathematical expression of equilibrium between nitrogen and phosphoric acid in plants. Science n.s. **84**: 422–423. 1936.
6. ————. Foliar diagnosis: Principles and practice. Plant Physiol. **12**: 571–599. 1937.
7. ————. Foliar diagnosis: The relation to the optimum nutrition of the potato. Plant Physiol. **13**: 677–694. 1938.
8. ————, and MACK, WARREN B. Foliar diagnosis in relation to development and fertilizer treatment of the potato. Jour. Agr. Res. **57**: 397–414. 1938.

THE RELATION OF CABBAGE HARDINESS TO BOUND WATER, UNFROZEN WATER, AND CELL CONTRACTION WHEN FROZEN[1]

J. LEVITT

(WITH ONE FIGURE)

Several investigators have attempted to correlate bound water with hardiness in cabbage. ROSA (**18**) was the first to discuss the point. He measured total ice in hardened and unhardened plants by means of the dilatometer. Greater quantities of water remained unfrozen in the former, even though total moisture content was less. He further found that hardened tissue dried more slowly at 60° C. than did tender tissue. BOSWELL (**1**) confirmed this. But ROSA's results, however suggestive, tell nothing about colloidally bound water. At the relatively high temperatures he used (mainly −5° C.) most of the unfrozen water must have been held by osmotic forces, and the difference between hardened and unhardened was at least mainly due to the greater concentration of the former's sap. The oven-drying results may quite readily be explained by a less water-permeable cuticle in the hardened, as indeed the more waxy appearance seems to indicate is the case.

KIMBALL (**11**), using NEWTON and GORTNER's cryoscopic method, found that the percentage of bound water usually varies inversely with hardiness.

GREATHOUSE (**5**), with the same method, obtained a direct correlation between bound water and hardiness. Statistical analysis of his results led him to conclude that bound water and osmotic pressure are not closely correlated, and that hardiness is more closely correlated with the former than with the latter. Nonetheless, it is highly probable that the increase in bound water is at least partly due to that bound by sugars, which may be present in larger quantities even in plants with lower total osmotic pressure. Furthermore, table I shows that when calculated per gm. of dry matter, the amount was not any greater in the hardened than in the unhardened.

DUNN (**3**) determined the dye adsorption of cabbage juice and took this as a measure of the relative quantity of hydrophilic colloids, presumably on the assumption that dye adsorption and water adsorption are correlated. The average amount of dye adsorbed was higher in the hardened than in the unhardened. Recently (**4**) he has concluded that the method is not very reliable as a measure of hardiness.

LEVITT and SCARTH (**13**), using a plasmometric method, were unable to detect any non-osmotically bound water in either hardened or unhardened

[1] Investigation carried out at the Division of Plant Physiology, University of Minnesota, under a Royal Society of Canada Fellowship.

TABLE I

BOUND WATER OF UNHARDENED AND HARDENED CABBAGE (VARIETY, EARLY JERSEY WAKEFIELD). ADAPTED FROM GREATHOUSE

TREATMENT	CONCENTRATION BY REFRACTOMETER	BOUND WATER	BOUND WATER PER GM. DRY MATTER
	%	%	*gm.*
15% soil moisture	8.2	6.53	0.80
14% " "	8.4	7.90	0.94
Periodically wilted	11.0	9.53	0.87

cabbage. There are two objections to these results. The lack of sensitivity of the method may conceivably fail to reveal a binding of 2–3 per cent. of the total water; and since the determinations were made at room temperature, the hydration would be considerably less than that obtained by the other methods (except the dye adsorption test).

Of the numerous methods used for measuring bound water, the consensus of opinion now seems to favor the calorimetric (SAYRE **19**, MEYER **15**, etc.). It has one outstanding advantage which is usually overlooked —the material is tested in the normal, living condition, and therefore gives a measure of total bound water. There is no loss due to coagulation of the proteins as is the case with any procedure which kills the tissues (*e.g.* the cryoscopic and probably the dilatometric.). Furthermore, it is amenable to at least as high a degree of accuracy as any, and is free from complications such as arise when foreign solutes are added as in the cryoscopic method.

However, as heretofore used, the calorimetric method (as well as the dilatometric) measures total bound water—that held colloidally, osmotically, and by hydration of sugars. Consequently, differences in quantity may be due to any or all of these three. DEXTER (**2**), in fact, found that after standing for 40 hours in 10 per cent. sucrose, 2.5 per cent. sucrose, and water respectively, cabbage leaves contained quantities of bound water in that order.

In the following investigation a modification was introduced which enabled the separate calculation of osmotically and non-osmotically bound water. LEBEDINCEV (**12**) made use of a similar correction for the dilatometric method.

Methods and results

MÜLLER-THURGAU (**16**) first evolved the calorimetric technique for determining the quantity of ice in frozen plant material. His technique is now regarded as too crude, though his results are probably sufficiently accu-

rate, since he compensated for lack of sensitivity in his apparatus by use of large samples (100 gm.).

The technique employed naturally depends on the kind and quantity of material used. MEYER (15) filled metal, rubber-stoppered tubes with 20-gm. samples of pine needles and froze these by immersion in a salt solution at the required temperature. SAYRE's (19) method is essentially the same. When freezing was complete, the tube was removed, unstoppered, and the needles prodded out into the calorimeter.

This was found unsuitable for cabbage, mainly on account of its high moisture content. Some water was always lost by condensation on the inside of the tube. There was sometimes a tendency for the material to stick to the walls and this increased the time required for transfer, which even under the best conditions is long enough to allow a temperature change.

ROBINSON (17) using much smaller samples (0.4 to 0.8 gm.) wrapped the material in tinfoil, which was placed in a weighing bottle and frozen in air. Transfer to the calorimeter—tinfoil and all—was in this way accomplished with maximum rapidity.

When using large samples, however, this method has its disadvantages. In the case of water or solutions, the tinfoil is burst due to the expansion, and there may be some loss of material. At the high freezing temperatures used in the following investigation, enclosure of the wrapped sample in a weighing bottle was found very favorable to undercooling. Because of these difficulties, a new type of container was adopted.

A cylindrical cup (A, fig. 1), 4 inches long by 0.75 inch in diameter, together with a close-fitting cap are constructed from sheet brass (0.02″ to 0.035″ shimstock). The lid has a flanged edge which allows it to slip easily over the cup, and yet when squeezed in place it makes a water-tight contact with a collar near the top of the cup. Before the container is soldered together all the pieces are weighed. This allows an exact calculation of the specific heat of the finished container since those of the brass and the solder are known (0.09 and 0.045 respectively). The capacity is about 25 cc.

The advantages of these containers are several. They are unbreakable. They are good conductors of heat and therefore allow temperature equilibria to be reached in minimum time. Their specific heat is small and is exactly known. They are water-tight and therefore do not require enclosure in a weighing bottle which would greatly favor undercooling. (When filled with a sample and left in the chamber at 0° C. with the full force of the fan on them for 24 hours, they lost not more than 1 mg. in weight.) When used correctly (see below) they are never burst by ice formation. Their transfer from the chamber to the calorimeter is instantaneous and therefore no appreciable temperature change occurs.

The samples were frozen in a cold chamber equipped with a fan and thermoregulator. The temperature fluctuated not more than $\pm 0.4°$ C. This range of variation was narrowed by use of a double-walled can retained inside the chamber. The space between the two walls was filled with salt solution, and the inner can was covered with a lid perforated to accommodate a thermometer. The temperature of the air in the inner can kept constant to $\pm 0.1°$ C. A pair of tongs was kept in the chamber, with which to handle the samples and the lid of the can.

Even pure water may remain unfrozen for 12 or more hours at a few degrees below the freezing point. Supercooling was prevented by maintaining the cups slightly agitated, in the following way. A square of wire screen was tied to a metal ring on a retort stand and a cardboard "sail" suspended below the screen. The force of the fan on this sail was sufficient to keep the screen (together with the samples placed on it) in constant

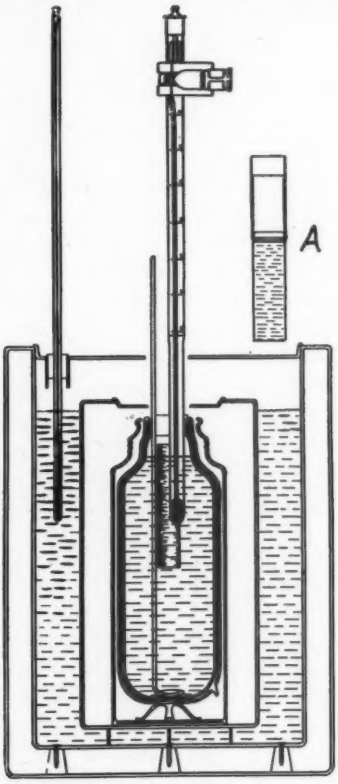


FIG. 1. Design of cup (A) and calorimeter used in the determinations.

gentle motion. When frozen, the samples were transferred from the screen to the can and left for two or three hours to come to equilibrium.

A pint thermos bottle fitted with a stopper, Beckman thermometer, and stirring rod served as the calorimeter (fig. 1). Into it were pipetted 400 cc. of distilled water at a standard temperature (in this investigation 21.4 ± 0.2° C.). Since even a thermos bottle is not perfectly insulated, it was necessary to retain it in an atmosphere of as nearly constant temperature as possible. This was accomplished by means of a triple-walled can. Between the outer two walls was a dead-air space, while water was retained between the inner two. By adjusting the temperature of the water, the air in the innermost can (which contained the thermos bottle) was readily maintained at a constant temperature (0.9° ± 0.15° C. above the equilibrium temperature attained by the calorimeter after the frozen sample had been added). The sample used was such as to cause a 2.5° C. (± 0.15° C.) drop in the temperature of the calorimeter. All the thermometers in use were calibrated against a precision thermometer.

Calibration of Calorimeter

About 10 gm. of water were accurately weighed in the tared containers. The cups were then laid on their sides on the metal screen inside the cold chamber. (If stood up, the cups either burst or the back pressure of the cup walls prevents complete freezing.) After 12 or more hours, they were transferred to the double-walled can inside the cold chamber and allowed a further three hours to come to equilibrium.

The calorimeter itself was set up at the same time and allowed three hours to come to equilibrium with the air in the constant temperature can. Its temperature was then read and a cup quickly transferred to it. After half an hour, stirring was commenced and continued until the temperature became constant (5 to 10 min.). The end point was readily distinguished as it remained constant (to 1/400 °C.) for 10 or more minutes.

For each following determination the calorimeter must be emptied, wiped dry, and refilled with water at the required temperature. It is then allowed about 10 min. to reach equilibrium.

The calibration factor (F) is calculated from the following formula (using Meyer's symbols):

$$F = \frac{W_w S_i T_s + W_w S_w T_e + W_c S_c (T_e + T_s) + W_w Q}{N S_w (T - T_e)}$$

where W_w = wt. water
W_c = " cup
S_i = sp. heat ice (0.50—see Sayre)
S_w = " " water (1.004—see Sayre)
S_c = " " cup (0.08–0.085)

T = temp. of calorimeter at start
T_e = " " " " end
T_s = " " sample (*i.e.* + °C. below 0)
Q = Heat of fusion of ice in calories (79.71)
N = Vol. water in calorimeter (cc.)

The results were as follows:

TABLE II

CALIBRATION OF CALORIMETER WITH WATER FROZEN AT −5.6° C.

W_c	W_w	TIME FROZEN	$T-T_e$	T_s	TEMPERATURE OF CAN	FACTOR
gm.	*gm.*	*hr.*	*°C.*	*°C.*	*°C.*	
6.932	9.821	18	2.4175	18.6725	19.6	1.039
8.285	9.799	19	2.425	18.97	19.75–19.8	1.040
7.125	9.8175	20	2.4275	19.095	19.9 –19.95	1.039
8.640	9.856	21	2.4475	18.89	19.9	1.036
					Av.	1.0385 ± .002

DETERMINATIONS WITH PURE SOLUTIONS

As a test of the method, measurements were first made in the complete absence of colloidally bound water. The amount of ice formed was compared with the quantity that should arise as estimated from the freezing point (T_m) of the solution ($W_f = W_w - \frac{T_m}{T_s} \times W_w$). A modification of MEYER's formula for free water was used to avoid the necessity of knowing the value of Q at the freezing point. The ice is assumed to thaw at 0 °C. (HAMPTON and MENNIE [8]). This gives exactly the same result as MEYER's formula and it has the advantage of simplicity.

$$W_f = \frac{FNS_w\ (T - T_e) - [\,(T_e + T_s)\ (W_d S_d + W_w S_w + W_c S_c)\,]}{Q - 0.5T_s}$$

where W_d = wt. of dry matter
S_d = sp. heat of dry matter
S_b = sp. heat of bound water (assumed = S_w)

Other symbols as in the previous formula.

These results indicate about 5 mols water bound per mol sucrose (table III).

According to SCATCHARD (21), sucrose is hydrated probably with six molecules of water, and a solution is a mixture of water, anhydrous sucrose, and hydrated sucrose. His figures for the average amount bound at 0° C. range between 6 molecules for the most dilute and 3.8 molecules for the most concentrated solutions. Since the concentration of the solution in table III was between these two extremes, the value of five molecules of water per molecule of sucrose agrees well with SCATCHARD's calculations.

TABLE III

$\frac{M}{2}$ SUCROSE FROZEN AT −5.6° C.

FROZEN	W_w	W_d	W_c	$T-T_e$	T_e	T_m	W_f	W_b	$\frac{W_b}{W_d}$
hr.	*gm.*	*gm.*	*gm.*	*°C.*	*°C.*		*gm.*	*gm.*	
20	12.725	2.185	8.503	2.65	18.73	1.025	9.891	0.619	0.283
21	12.717	2.184	7.121	2.6375	18.7525		9.8695	0.637	0.292
22	12.746	2.189	6.929	2.665	18.88		9.991	0.516	0.236
23	12.697	2.180	8.277	2.6575	18.7675		9.940	0.531	0.244
								Av. ...	0.264

DETERMINATIONS WITH CABBAGE SEEDLINGS

Before applying the method to measure bound water in cabbage seedlings it is first necessary to find the specific heat of the dry matter which constitutes the tissues. This, however, involves difficulties, for it is essential that the same standard conditions prevail (including the 2.5° temperature drop of the calorimeter). The following method was finally adopted:

New cups were made with dimensions similar to the above-described standard cups, save that they were hemispherical in cross section and fitted inside these. After filling with weighed quantities of dried cabbage tissue the hemi-cups were sealed (by soldering) and placed inside the standard cups. Enough water (about 9.3 cc.) was now pipetted into the standard cups to enable the whole when frozen to cause the usual 2.5° C. drop in the temperature of the calorimeter. The specific heat of the hemi-cups was 0.07. It is essential that the hemi-cups be perfectly sealed, otherwise the water penetrates and invalidates the results.

In order to calculate the non-osmotically bound water, one must know the total moisture and the freezing point of the tissues. To eliminate the

TABLE IV

SPECIFIC HEAT DETERMINATIONS WITH DRIED CABBAGE PETIOLES AT −5.6° C.

W_c	W_t HEMI.–CUP	W_w	W_d	$T-T_e$	T_e	SPECIFIC HEAT
gm.	*gm.*	*gm.*	*gm.*	*°C.*	*°C.*	
8.2815	11.5207	9.1625	6.4245	2.415	18.995	0.251
6.929	10.6095	9.1575	6.451	2.40	18.975	0.242
8.2815	11.5207	9.166	6.4245	2.4225	18.9025	0.275
7.122	11.5207	9.159	6.4245	2.4075	18.8225	0.263
8.466	10.6095	9.186	6.451	2.41	18.8625	0.237
					Av.	0.254 ± .02

error of sampling all three tests were made on one and the same sample in the following way:

After the calorimetric determinations, the cups were transferred to a vacuum oven and dried to constant weight at 60° C. (to prevent caramelization which would occur at 100° C.). Sufficient water was then added to restore the original weight of the fresh tissue. After storing for two days at +2° C., to enable the dried tissue to soak up the water, the juice was expressed and the freezing point determined. Thus total moisture, total ice, and freezing point were all determined on the same sample.

An objection to this method of determining the freezing point is that a certain amount of hydrolysis of disaccharides may occur during the drying. However, since most of the sugars present in cabbage are monosaccharides (HARVEY, 9) the error cannot be very great. An idea of the extent of error was obtained by comparing the freezing point obtained in this way with that of a duplicate sample whose juice was expressed and tested immediately after thawing. Both sets had been frozen 24 hours at −8° C., a temperature sufficient to kill the tissues.

	VACUUM DRIED	CONTROL
Unhardened	−0.94 °C.	−0.92 °C.
"	−0.88	−0.81
Hardened	−1.35	−1.31
"	−1.12	−1.14

Judging from these figures, the error introduced is slight, probably less than the variation between samples.

Knowing W_f at temperature T and subtracting this from total water, the result obtained is the total "bound water" at this temperature—osmotic plus non-osmotic. Separate determinations of these two constituents are possible on the prevalent assumption that the non-osmotically bound water is that portion which does not act as a solvent, *i.e.*, which does not obey the laws for ideal solutions.

$$\begin{aligned} \text{If } & W_w = \text{total water} \\ & W_f = \text{frozen water} \\ & W_o = \text{osmotically bound water} \\ & W_b = \text{non-osmotically bound water} \\ \text{Then } & W_w = W_f + W_o + W_b \\ \text{But } & W_o = \frac{T_m}{T_s}(W_w - W_b) \\ \text{Therefore } & W_b = W_w - \frac{W_f}{1 - \frac{T_m}{T_s}} \end{aligned}$$

These quantities were determined for unhardened and hardened (2 weeks at +5° C. with continuous light) cabbage plants when T = 5.6° C. below zero (table V). Only the petioles were used as these pack easily into the cups. Each sample contained the petioles of about a dozen plants.

TABLE V

UNHARDENED CABBAGE FROZEN AT −5.6° C.

TIME FROZEN	MOISTURE	W_w	W_d	W_c	$T - T_e$	T_e	T_m	W_f	$\frac{W_f}{W_d}$	$\frac{W_f}{W_w + W_d}$	$\frac{W_w - W_f}{W_d}$	W_b	$\frac{W_b}{W_d}$
hr.	%	*gm.*	*gm.*	*gm.*	°C.	°C.		*gm.*				*gm.*	
15	92.0	12.376	1.076	6.181	2.7225	18.89	0.80	10.563	9.84	0.79	1.68	0.051	
26	93.0	12.201	0.918	6.685	2.695	18.565	0.802	10.523	11.50	0.80	1.83	−0.087	
38	92.2	12.176	1.028	6.181	2.6525	18.3575	0.842	10.340	10.09	0.79	1.78	0.011	
23	91.3	11.635	1.102	8.277	2.585	19.2375	0.72	9.927	9.03	0.78	1.55	0.238	
25	91.8	11.668	1.044	6.929	2.6125	19.0675	0.68	10.146	9.75	0.80	1.46	0.120	
21	93.3	11.638	0.840	6.181	2.6075	18.9325	0.715	10.186	12.16	0.82	1.73	−0.057	
22	92.2	11.502	0.991	6.685	2.585	19.305	0.715	10.023	10.14	0.80	1.49	0.008	
23	92.5	11.592	0.946	8.635	2.6125	19.16	0.725	10.106	10.71	0.81	1.57	−0.024	
								Av.	10.40	0.80	1.64	0.032	0.03
HARDENED CABBAGE FROZEN AT −5.6° C.													
16	89.0	12.759	1.577	8.463	2.63	18.98	1.23	9.810	6.24	0.69	1.87	0.189	
25	88.0	12.600	1.724	8.463	2.56	19.52	1.287	9.371	5.45	0.66	1.87	0.430	
27	86.8	12.455	1.895	8.503	2.4675	18.475	1.392	9.091	4.81	0.63	1.77	0.356	
22	87.6	12.483	1.762	8.463	2.5825	18.9225	1.16	9.684	5.51	0.68	1.59	0.271	
24	87.2	12.394	1.826	7.121	2.5275	19.2375	1.215	9.350	5.14	0.66	1.67	0.453	
								Av.	5.43	0.66	1.75	0.340	0.20

The most striking difference between the two sets of cabbages is in the quantity of ice formed, almost twice as much per gm. dry matter $\left(\frac{W_f}{W_d}\right)$ in the unhardened as in the hardened (10.40 and 5.43 gm., respectively). This is obviously due to the difference in the total moisture and the freezing point, both of which are lower in the hardened.

Expressed on the basis of fresh weight, $\left(\frac{W_f}{W_w + W_d}\right)$, the non-hardened contained 0.80 gm. ice per gm. of tissue, while the hardened had only 0.66 gm. This means that if the ice formed extracellularly, the unhardened cell was reduced to one-fifth its original volume, the hardened only to one-third.

The total unfrozen water per gm. dry matter, $\left(\frac{W_w - W_f}{W_d}\right)$, is slightly greater in the hardened than in the unhardened (1.75 and 1.64 respectively).

The non-osmotically bound water has a very small value in both cases, though considerably larger in the hardened plants—0.20 gm. per gm. of dry matter as compared to 0.03 gm. in the unhardened.

MEASUREMENTS AT CRITICAL FREEZING TEMPERATURES.—In order to apply results of this type to frost resistance it is essential to obtain measurements of the above quantities not at a random freezing temperature but at the two points just low enough to cause the same degree of injury (*e.g.* 50–75 per cent.) in hardened and unhardened plants respectively, *viz.*, at the critical freezing temperature of each.

The results already recorded in table V were obtained at the critical freezing temperature for hardened plants. Table VI shows the injury that

TABLE VI

INJURY TO HARDENED CABBAGE FROZEN AT −5.6° C. EACH VALUE AN AVERAGE OF NINE PLANTS

GROUP	TIME FROZEN	INJURY
	hr.	%
1	9	55
2	16	45
3	20	50

resulted. Unhardened plants, on the other hand, suffer as much at considerably higher temperatures (table VII). It should be emphasized that the plants tested for hardiness were in all cases taken from the same group and frozen at the same time as those tested calorimetrically.

When calorimetric determinations were made at −5.6° C., control unhardened plants were simultaneously tested for resistance to this temperature, always with the expected result, 100 per cent. injury.

TABLE VII

RESISTANCE OF UNHARDENED CABBAGE SEEDLINGS. EACH VALUE AN AVERAGE OF EIGHT PLANTS

GROUP	TEMPERATURE	TIME FROZEN	INJURY
	°C.	hr.	%
1	−2.7	10	100
2	−2.1	11	85
3	−1.4	16	25

The critical temperature for the unhardened is apparently about −1.8° C. The calorimetric determinations were made at −2.1° C. (table VIII). At this relatively high temperature it was found necessary to inoculate the cups of petioles with an ice crystal in order to insure freezing. The potted plants, however, froze readily, apparently on account of their thin leaves, which were in constant gentle motion due to the force of the fan.

A comparison between these values for unhardened plants at their critical freezing temperature (−2.1° C.) and those already obtained for hardened plants at theirs (−5.6° C.) yields several important facts.

First, although the amount of ice formed per gm. of dry matter is about 75 per cent. greater in the unhardened than in the hardened cells (9.43 and 5.43 gm. respectively), the former retain 3.5 times as much water in the liquid state as do the latter (6.30 and 1.75 gm. per gm. of dry matter respectively). Even on the basis of fresh weight (though this is not shown in the table) there is twice as much water per gm. in the unhardened as in the hardened (0.40 and 0.21 gm. respectively). There can, therefore, be no question of a reduced degree of dehydration causing the increased frost resistance.

Second, the unhardened contain only 0.56 gm. ice per gm. of fresh weight though suffering 85 per cent. injury, while the hardened have 0.66 gm. and show only 50 per cent. injury. This means that if the ice formed extracellularly, most of the unhardened cells are killed when contracted roughly to half their original volume, the hardened cells only after a shrinkage to one-third. (The greater specific gravity of the hardened will only tend to enhance this relationship.)

Finally the quantity of non-osmotically bound water per gm. of dry matter is greater in the unhardened than in the hardened (0.78 and 0.20 gm.).

It is important to establish whether the injury really is caused by extracellular ice formation as assumed above. Two other possibilities exist: the damage may occur as a result of intracellular ice formation or it may arise during thawing. Since in the above tests both freezing and thawing were rapid, the effect of slowing up these processes was next investigated.

TABLE VIII

UNHARDENED CABBAGE FROZEN AT −2.1° C.

TIME FROZEN	MOISTURE	W_w	W_d	W_c	$T - T_e$	T_e	T_m	W_f	$\frac{W_f}{W_d}$	$\frac{W_f}{W_w + W_d}$	$\frac{W_w - W_f}{W_d}$	W_b
hr.	%	*gm.*	*gm.*	*gm.*	°*C.*	°*C.*		*gm.*				*gm.*
42	94.1	14.1135	0.879	7.116	2.4475	18.205	0.762	8.448	9.61	0.56	6.46	0.872
43	94.0	14.1095	0.8975	6.173	2.4625	18.6025	0.762	8.475	9.44	0.56	6.28	0.825
44	93.9	14.0985	0.9145	6.680	2.47	18.85	0.805	8.457	9.25	0.56	6.17	0.392
								Av. ...	9.43	0.56	6.30	0.70

Two sets of unhardened cabbages were placed in the chamber at $-0.5°$ C. and the temperature was gradually and uniformly lowered during a three-hour period to $-2.1°$ C. (*i.e.*, 0.5° per hr.). This necessitated hand regulation, the temperature varying not more than $\pm 0.2°$ C. Just when ice formation began it is impossible to say, but it was sufficient to be observed at $-1.5°$ C.

A third set of plants was placed in the chamber at $-2.1°$ C. Fifteen hours later this fast-frozen set and one of the slow-frozen were removed and immediately thawed at room temperature. The other slow-frozen set was thawed slowly in the cold chamber as follows: the temperature was allowed to rise to $-1.1°$ C. during 15 min., at which it was retained for one hour. During the next 20 min. it rose to $+1°$ C., and 20 min. later the plants were removed from the chamber. In this way thawing occurred during a two-hour period as compared with the others which thawed completely in five minutes.

Table IX shows that neither slow freezing nor slow thawing caused any reduction in injury.

TABLE IX

UNHARDENED CABBAGE FROZEN 15 HOURS AT $-2.1°$ C. EACH RESULT AN AVERAGE OF EIGHT PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	45
Slow	Fast	75
Slow	Slow	80

An older set of unhardened plants, which owing to their stunted growth were sufficiently hardy to have their critical freezing temperature lowered to $-3.1°$ C. was similarly tested. This time slow freezing was begun with 1 hr. at $-1.2°$ C., followed by 1.5 hr. at $-2.2°$ C. (after a half hour at this temperature freezing was first noticeable). Slow thawing was induced during a three-hour period in which the temperature gradually rose to $+3.5°$ C. Here again, no protective effect was produced by reducing the rate of freezing or thawing (table X).

TABLE X

UNHARDENED (SLIGHTLY HARDY) CABBAGE FROZEN 8.5 HOURS AT $-3.1°$ C. EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	90
Slow	Fast	65
Slow	Slow	95

Fully hardened plants were next tried. The temperature was dropped 1° per hour, from −3.3° C. to −7.3° C. At the end of the first hour almost all were frozen; at the end of the second hour all were frozen stiff. For slow thawing the temperature was raised about 1° per hour for 5 hours, at the end of which the temperature was about 0° C. and the plants were removed.

TABLE XI

HARDENED CABBAGE FROZEN FOR 15 HOURS AT −7.3° C.
EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	100
Slow	Fast	80
Slow	Slow	80

The same experiment was repeated on similarly hardened plants at a slightly higher temperature (−6.3° C.). For slow freezing, the temperature was dropped 1° C. per hour, from −2° C. to −6.3° C. The first trace of freezing was observable at the end of one hour; after the second hour half were frozen. Slow thawing took 4.5 hours at the end of which the temperature was +1° C.

TABLE XII

HARDENED CABBAGE FROZEN FOR 17 HOURS AT −6.3° C.
EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	90
Slow	Fast	25
Slow	Slow	15

In both cases the slow freezing reduced the injury somewhat, in neither did the slow thawing have any effect.

Consequently, it appears that under the freezing condition employed, the injury suffered by both the hardened and unhardened plants occurred during freezing and not during thawing. Further, the unhardened were not protected by reducing the rate of ice formation. In the case of the hardened, some protection did result from slowing up the rate of freezing. It seems probable then, that the hardened plants can withstand a cell shrinkage to even less than $\frac{1}{3}$ of its original volume (which was stated above to be the limit of its endurance).

Discussion

Before discussing the significance of these figures, it is essential to know how dependable they are. The values for total ice, and the various ways of expressing them, appear quite reliable. On the other hand, the estimation of that portion of the unfrozen water that is non-osmotically bound is open to several sources of error and since the quantity is so small, it can be markedly altered by any of these, a fact which is attested to by the decided variation in the results.

The great difference between this value when determined at $-5.6°$ C. and at $-2.1°$ C. for unhardened plants can be due only to error. The slight temperature effect together with the greater but oppositely acting concentration effect can account for only a small fraction of the difference.

One source of error that may explain another portion of this difference is the fact that the freezing point of a solution is not exactly proportional to the concentration, as is assumed in the formula for W_b.

A slight error in the value for the freezing point of the juice (which as already pointed out probably does occur) may greatly alter the value of W_b.

Furthermore, the assumption is made that the average specific heat of the dry matter and the water is the same as that of the two when mixed. Here again, the slight error involved may affect W_b.

It should be emphasized that of all these sources of error, only the last can have any influence (and that a negligible one) on the various values for W_f.

As a check on the reliability of the values obtained, it is interesting to compare them with those found by other investigators. On the basis of total water, the unhardened contained only 0.3 per cent. at $-5.6°$ C., 5 per cent. at $-2.1°$ C., while the hardened had 2.7 per cent. at $-5.6°$ C. These quantities are somewhat lower than those obtained by KIMBALL (**11**) mainly 3–6½ per cent.) and by GREATHOUSE (**5**) (5–9 per cent.) both of whom used the cryoscopic method. However, KIMBALL certainly, and GREATHOUSE probably, used NEWTON and GORTNER'S original formula without GROLLMAN'S (**7**) correction (which was approved by GORTNER and GORTNER, **6**). This correction always yields lower values than the original formula, and therefore would bring the results of the above workers into closer agreement with our own.

The results show that at any given temperature the hardened plants may have less water withdrawn in the form of ice. But (1) at a temperature which causes practically complete killing of unhardened plants, they retain in their tissues 3.5 times as much water in the unfrozen state as do the hardened at the temperature required to produce the same amount of injury in them; (2) the amount of non-osmotically bound water in hardened plants is very small, and though larger than that for unhardened plants at the same

temperature (−5.6° C.), it is nevertheless smaller when the two groups are compared at their respective critical freezing temperatures; (3) at their respective freezing temperatures more ice is formed per gram fresh weight in the hardened than in the unhardened plants.

Cabbage hardiness is therefore not determined simply by resistance to dehydration (either of the tissue as a whole, or of the colloidal constituents, *viz.*, the protoplasm in particular), or to the amount of ice which forms, but by some increased ability to resist the effects of freezing.

These facts on the other hand in no way oppose the theory put forward by SCARTH and LEVITT (**20**). The latter consider that one factor in frost injury is the protoplasmic strain described by ILJIN (**10**), and that the greater resistance to plasmolysis and deplasmolysis injury which they found in hardened plants is due to a greater resistance to the injurious effects of the strain.

This conception is fully borne out by the fact that cells of hardened plants must be subjected to a contraction to one-third (or even less) of their normal volume before injury sets in, those of unhardened plants to only one-half their normal volume. This may be interpreted as the result of greater resistance to protoplasmic strain in the former, though only to that which occurs during freezing, since rate of thawing has no effect on the degree of injury.

Another hardiness factor according to this theory is permeability. Since the rate of freezing is of no detectable importance insofar as unhardened plants are concerned, and since ice forms inside the cell only as a result of rapid freezing, intracellular ice apparently does not arise at the temperatures and the rates of freezing to which they were subjected. Permeability is therefore not the limiting factor in the frost injury of unhardened plants.

Hardened plants, on the other hand, are less injured by slow than by rapid freezing, an indication that intracellular ice is involved, and that the permeability of the hardened cells is insufficient to prevent it. But how does this agree with the fact that hardened cells are more permeable than unhardened (LEVITT and SCARTH, **13**)?

Agreement appears as follows: When subjected to its critical freezing temperature the unhardened plant is only 1⅓° below its freezing point. Under this small gradient ice formation is relatively slow. The permeability rate (even that of unhardened plants) is sufficient to keep up with it and so to permit extracellular ice formation. In this case, however, protoplasmic strain kills the cells.

On account of its greater resistance to protoplasmic strain, the cell, after hardening, is now able to withstand a lower temperature. But this increases the gradient between the temperature and its freezing point, until at the critical freezing temperature the two are 4.5° C. apart. The gradient is

now more than three times as large as in the case of the unhardened cells when subjected to their critical freezing temperature, and therefore overbalances the greater permeability (which is only about twice that in the unhardened cells (LEVITT and SCARTH, **13**). Furthermore, on account of the lower temperature, the permeability is decreased (VAN'T HOFF coefficient is 2.2). It is, therefore, not surprising that the exosmosis of water from the cell is no longer able to keep up with the rapid ice formation, and intracellular ice begins to form.

Instead of resistance to protoplasmic strain being the limiting factor, as when the cabbage cell is in the unhardened state, it is replaced by the other factor—permeability—which is now insufficiently high. With a more gradual temperature drop the situation is reversed. Intracellular ice formation is prevented and a lower temperature is withstood, until the protoplasmic strain (which is now once more the limiting factor) is increased sufficiently to injure the cell.

This may well describe the situation under artificial conditions, where the unhardened plant is suddenly exposed to its critical freezing temperature and the hardened plant to its own, which is 3.5° C. lower. In nature, of course, such a difference does not happen. The temperature drop is gradual. How then can the same explanation hold true?

There are two cases in which the plant may behave in essentially the same way as under these artificial conditions. In still air supercooling is considerable, so that even though the temperature drop may be gradual, it is quite possible for ice formation to begin suddenly at several degrees below the freezing point. Also, when the sun sets, or even if suddenly clouded over, the plant which had been thawed by it is now quickly exposed to a temperature considerably below the freezing point. These will afford a similar state of affairs to those provided in artificial freezings.

Summary

Calorimetric determinations of ice formation in frozen cabbage petioles were combined with determinations of the freezing point and the total moisture. This not only gave a measure of frozen and unfrozen water, but also allowed a separate estimation of osmotically and non-osmotically bound water.

The results were as follows:

I. At – 5.6° C.:

1. A test of the method indicated 5 mols of water bound per mol sucrose in a half molar solution.

2. About twice as much ice per gm. of dry matter occurs in the unhardened as in the hardened cabbages (10.40 and 5.43 gm. respectively).

3. If the ice formed extracellularly, the unhardened cells contracted to one-fifth of their volume, the hardened only to one-third.

4. The hardened plants retained a slightly larger quantity of unfrozen water per gm. of dry matter than did the unhardened (1.75 and 1.64 gm. respectively).

5. The non-osmotically bound water is very small in both, though larger in the hardened than in the unhardened (0.20 and 0.03 gm. per gm. of dry matter respectively).

II. When compared at their critical freezing temperatures (−5.6° C. for hardened, −2.1° C. for unhardened):

1. Unhardened tissue retained 3.5 times as much water (in the liquid state) per gm. of dry matter as did hardened (6.30 and 1.75 gm. respectively). Injury due to dehydration is therefore precluded.

2. The unhardened cell was contracted only to one-half its volume, the hardened to one-third. This indicates the greater resistance of the latter to protoplasmic strain (assuming this to be the cause of injury).

3. The quantity of non-osmotically bound water is greater in the unhardened than in the hardened plants (0.78 and 0.20 gm. per gm. of dry matter respectively).

4. Reducing the rate of freezing and thawing did not alter the critical freezing temperature of the unhardened plants—an indication that injury was due to the cell contraction and not to intracellular ice nor to rapid thawing. With hardened plants, on the other hand, the critical freezing temperature was lowered by reducing the rate of freezing (but not the rate of thawing). This indicates that if intracellular ice-formation was prevented the cells of hardened plants were even more resistant to contraction injury than mentioned above.

The author wishes to express his thanks to Dr. R. B. HARVEY and Dr. R. H. LANDON of the Division of Plant Physiology, University of Minnesota, for their constant cooperation and aid, without which this research would not have been possible; to Prof. G. W. SCARTH of McGill University for his many helpful suggestions during the writing of the manuscript; to Dr. J. H. MENNIE of McGill University for his elucidation of the physico-chemical problems involved in this work; to Mr. F. T. CARTER, technician of the Division of Plant Physiology, University of Minnesota, for aid in constructing the apparatus used.

MCGILL UNIVERSITY
MONTREAL, CANADA

LITERATURE CITED

1. BOSWELL, V. R. Dehydration of certain plant tissues. Bot Gaz. 75: 86–94. 1923.

2. DEXTER, S. T. Growth, organic nitrogen fractions, and buffer capacity in relation to hardiness of plants. Plant Physiol. **10**: 149–158. 1935.
3. DUNN, S. Relation of hydrophilic colloids to hardiness in cabbage, brussels sprouts, and alfalfa plants as shown by the dye adsorption test. Plant Physiol. **8**: 275–286. 1933.
4. ————. Value of the dye-adsorption test for predetermining the degree of hardiness. Plant Physiol. **12**: 869–874. 1937.
5. GREATHOUSE, G. A. Effects of the physical environment on the physico-chemical properties of plant saps, and the relation of these properties to leaf temperature. Plant Physiol. **7**: 349–390. 1932.
6. GORTNER, R. A., and GORTNER, W. A. The cryoscopic method for the determination of "bound water." Jour. Gen. Physiol. **17**: 327–339. 1934.
7. GROLLMAN, A. The vapour pressures of aqueous solutions with special reference to the problem of the state of water in biological fluids. Jour. Gen. Physiol. **14**: 661–683. 1931.
8. HAMPTON, W. F., and MENNIE, J. H. Heat capacity measurements on gelatin gels. I. Canadian Jour. Res. **7**: 187–197. 1932.
9. HARVEY, R. B. Hardening process in plants and developments from frost injury. Jour. Agr. Res. **15**: 83–112. 1918.
10. ILJIN, W. S. Über den Kältetod der Pflanzen und seine Ursachen. Protoplasma **20**: 105–124. 1933.
11. KIMBALL, D. A. Effect of the hardening process on the water content of some herbaceous plants. Proc. Amer. Soc. Hort. Sci. **24**: 64–70. 1927.
12. LEBEDINCEV, ELISABETH. Untersuchungen über die wasserbindende Kraft der Pflanzen im Zusammenhang mit ihrer Dürre- und Kälteresistenz. Protoplasma **10**: 53–81. 1930.
13. LEVITT, J., and SCARTH, G. W. Frost-hardening studies with living cells. I. Osmotic and bound water changes in relation to frost resistance and the seasonal cycle. II. Permeability in relation to frost resistance and the seasonal cycle. Canadian Jour. Res., C **14**: 267–284; 285–305. 1936.
14. MAXIMOV, N. A. Experimentelle und kritische Untersuchungen über das Gefrieren und Erfrieren der Pflanzen. Jahrb. wiss. Bot. **53**: 327–420. 1914.
15. MEYER, B. S. Further studies on cold resistance in evergreens, with special reference to the possible rôle of bound water. Bot. Gaz. **94**: 297–321. 1932.
16. MÜLLER-THURGAU, H. Über das Gefrieren und Erfrieren der Pflanzen. II. Theile. Landw. Jahrb. **15**: 453–610. 1886.

17. Robinson, W. Free and bound water determinations by the heat of fusion of ice method. Jour. Biol. Chem. **92**: 699–709. 1931.
18. Rosa, J. T. Investigations on the hardening process in vegetable plants. Missouri Agr. Exp. Sta. Res. Bull. 48. 1921.
19. Sayre, J. D. Methods of determining bound water in plant tissue. Jour. Agr. Res. **44**: 669–688. 1932.
20. Scarth, G. W., and Levitt, J. The frost-hardening mechanism of plant cells. Plant Physiol. **12**: 51–78. 1937.
21. Scatchard, G. The hydration of sucrose in water solution as calculated from vapor-pressure measurements. Jour. Amer. Chem. Soc. **43**: 2406–2418. 1921.

EFFECT OF POTASH MANURING ON THE PRODUCTION OF ORGANIC MATTER[1]

OSCAR ECKSTEIN

(WITH TEN FIGURES)

Introduction

The formation of organic matter in the plant is the final result of the manifold chemical reactions and transformations of energy taking place during the course of the growing period. In the study of plant production pot and field experiments afford merely a means of determining the yields of vegetable matter, that is the cumulative effect of the many physiological processes which during the different stages of growth influence the development of the plant to a greater or lesser degree. Furthermore, the results of the usual type of fertilizer experiment only demonstrate the extent to which the amount of organic matter produced by the plant is dependent on the quantity and nature of the plant foods applied and on their joint functioning; but they can tell us little or nothing about the influence of manuring on the various synthetic processes in the plant, such as the formation of carbohydrates or of proteins.

Modern agriculture is not merely concerned with mass production but also aims at achieving a high standard of quality in its products. While this holds good for all branches of agriculture, it is particularly true in the case of modern grassland management and its ultimate products, *viz.*, meat and milk.

The maintenance of yields at a satisfactory level is of course important but the actual value of crops and particularly of the fodder produced, is determined in the main by its composition and more particularly by its content of digestible carbohydrates and proteins. Apart from the fact that reliable methods of estimating resorbable protein are still lacking, the ordinary fertilizer experiment both in the field and in pot cultures fails completely when it is a question of studying the effect of the nutrients applied on the quality of grassland produce.

That it is possible by suitable manurial treatment to influence selectively certain properties of Gramineae—take for example the strengthening of the straw due to potash and phosporic acid—is a well-known fact (**1**, **4**). On the other hand we have as yet been able to obtain but little insight into the relationship between the effects of fertilizers and the mechanism of plant production in general and the composition of the produce in particular. These relationships can be established more easily, when it is possible to

[1] Read at the Fourth International Grassland Congress, Aberystwyth, Wales, July 13–19, 1937.

obtain an accurate knowledge of the factors favoring the development of the particular property which it is desired to influence (**2**). It was therefore of interest to ascertain those physiological processes which predominate in determining the quality of the produce and how they can be influenced in the desired manner by manuring.

The most important process in the production of vegetable matter is the assimilation of carbon dioxide by the plant. In addition to making a careful study of the latter, we have also investigated the effect of potash on nitrogen metabolism, transpiration and chlorophyll formation.

All these vital processes are intimately connected with each other and a knowledge of the extent to which they may be influenced by manuring can be obtained only when it is possible to study them in one and the same plant sample under carefully controlled conditions. Moreover, conclusions of practical value can be drawn from the results obtained only when the plants used in such investigations have been grown under completely uniform environmental conditions, *i.e.*, at constant temperature, in uniform illumination, etc. It is obvious that plants grown in the open will not fulfill these requirements; for both in field and in pot experiments it is not possible to grow plants under conditions which remain constant over indefinite periods and can be reproduced at will. Grasses and cereals which are destined to serve as experimental material for comparative and quantitative studies of the influence of manuring on the assimilation of carbon dioxide and other vital processes, must be grown under artificial conditions with a minimum of variation, a requirement the practical fulfilment of which presents considerable technical difficulties. We have, however, been able to meet these requirements to a very considerable extent by the use of the apparatus constructed by GASSNER and GOEZE, a diagram of which is shown in figure **1** (**3**).

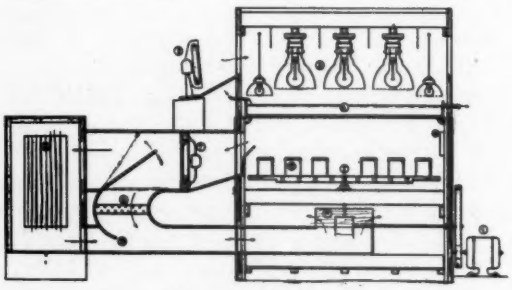


FIG. 1. Diagram of the apparatus.

1. Electric motor
2. Rotating wooden platform
3. Source of light
4. Shallow tank
5. Oscillating fan
6. Thermostat
7. Fans
8. Refrigerator
9. Electric heaters
10. Mixing canal
11. Air distributor
12. Pots for plants

In designing this apparatus particular care has been taken to insure uniform illumination. This has been achieved by mounting the pots containing the experimental plants on a mechanically-driven (1) rotating wooden disk (2) above which the source of light is situated. The source of light used (3) consists of a battery of Osram-Nitra lamps, some of which are fitted with Zeiss reflectors. A shallow tank with a glass bottom, through which water is kept constantly flowing (4), is interposed between the source of light and the plants and serves to absorb the heat radiated downwards from the lamps while allowing free passage to the light. The lamps themselves are protected from overheating by being constantly exposed to the draught created by an oscillating fan (5).

The temperature of the lower compartment, in which the plants are grown, is kept constant by means of a thermostat (6) and as the air is kept circulating continuously by means of two powerful fans (7), temperature variation is due merely to the slight deviation of the thermostat which, however, does not exceed $\pm 1°$ C. A third fan is connected to the evaporator of a refrigerator (8). Electric heaters (9) are used when higher temperatures are required. Cold and warm air are mixed in the canal (10) and the distributor (11) insures a uniform flow of air into the apparatus, at the same time preventing the current of air from directly striking the pots (12) containing the plants.

As a result of the slight deviation of the thermostat the relative humidity varies by about ± 5 per cent., while the variation in the CO_2 content of the air in the apparatus due to penetration of air from without does not exceed ± 5 per cent. of the CO_2 content per liter of air.

Figure 2 gives a side view of the interior of the apparatus, showing the pots in which the plants are grown.

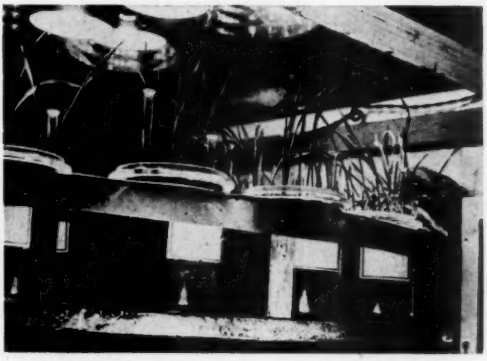


FIG. 2. Side view of the interior of the apparatus.

For technical reasons Gramineae have been selected as experimental plants. Wheat was used in most cases, as its comparatively broad leaves make it more suitable than the narrow-leaved grasses for this particular type of work. Rye, oats and barley have, however, also been shown to give satisfactory results. We have good reason to believe that the results obtained with these plants also hold good for meadow and pasture grasses.

The experimental plants were examined at four different stages of growth, *viz.*, after 13, 18, 25 and 30 days. The estimation of the absorption of carbon dioxide was carried out in the apparatus by GASSNER and GOEZE (8), cut leaves being used for this purpose. The method is based on the determination of the quantity of CO_2 absorbed by the surface of the leaf.

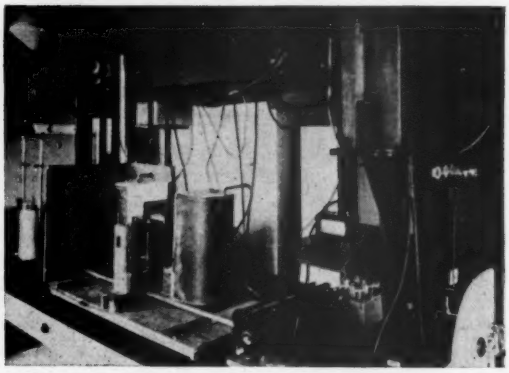


FIG. 3. The assembly for determining carbon dioxide assimilation.

The absorption of the carbon dioxide by the leaves takes place in the assimilation chamber. The latter consists of a narrow rectangular glass cell, which can be sealed by means of a metal cap. Through glass tubes fitted into the metal cap, air of known CO_2 content is allowed to flow at a constant rate from a large gasometer into the assimilation chamber. When the air emerges it is led into a small gasometer of 3 liters capacity standing over glycerol. From the latter the air is filled into evacuated flasks of known capacity and its CO_2 content estimated by the baryta method.

As the temperature must be kept constant during assimilation the cell containing the leaves is immersed in a water bath with glass walls, which is kept at 20° C. by a constant flow of cold water. The light necessary for the assimilation process is supplied by two 1000-watt lamps placed on either side of the assimilation chamber, so that both the upper and lower sides of the leaves receive the same amount of light.

Six leaves are used for each estimation and are fixed upright in a small glass boat with a mixture of lanolin and vaseline.

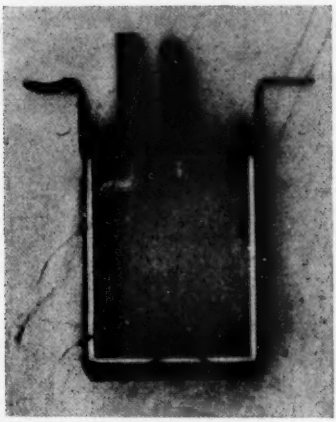


FIG. 4. Assimilation chamber closed (without assimilation frame).

The glass boat contains sufficient water to maintain the turgor in the leaves. The boat is weighed immediately before and after each estimation in order to determine the loss of water. The amount of CO_2 absorbed by the leaves from 3 liters of air in five minutes is taken as a measure of the actual

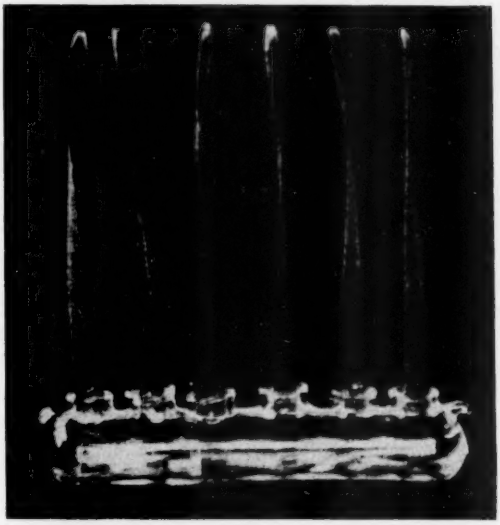


FIG. 5. Gramineae leaves in the glass boat during the estimation of assimilation and transpiration.

assimilation of CO_2 by the plant. Before the actual estimation is carried out the leaves are exposed to the light from both lamps and air is allowed to flow through the assimilation chamber for 20 minutes in order that the leaves may become adapted to the experimental conditions. Three estimations are then carried out at intervals of five minutes on each set of leaves. The length and breadth of the leaves are then measured accurately. From each set of values for the area of the leaves, the time and the amount of CO_2 absorbed, the quantity of CO_2 in milligrams absorbed by 100 sq. cm. of leaf surface in one hour is calculated and the mean of the three estimations taken.

The chlorophyll content of the leaves is estimated in the acetone extracts using a Zeiss gradient photometer and a light filter no. S. 66.613.5. The experimental extracts are not compared with a standard chlorophyll solution as for the purposes of our investigations it is sufficient to determine the relative variation of the values. In cases in which absolute values for the chlorophyll content of the leaves are required, these can be obtained by comparison with a standard chlorophyll solution. The protein and soluble nitrogen are estimated by the micromethods recommended by KEYSSNER and TAUBÖCK (**14**). The protein nitrogen is precipitated with trichloracetic acid and the nitrogen content of the precipitate and filtrate determined separately by the Kjeldahl method.

After preliminary tests we selected v. RÜMKER'S "Square Head" spring wheat, as the most suitable variety for our purposes. The wheat plants were grown in glass jars of 1-liter capacity, the substratum being glass sand, to which 1.5 per cent. purified peat mull had been added in order to improve its water-holding capacity. During the growth of the plants the sand-peat mixture was kept at 40 per cent. of its maximum water-holding capacity. Each pot received a dressing consisting of 20 mg. $MgSO_4 \cdot 7H_2O$, one drop of a 10 per cent. iron chloride solution and 25 mg. $CaCO_3$.

The experiments were conducted in two series, one with a low basal dressing consisting of 20 mg. N as $Ca(NO_3)_2$ and 20 mg. P_2O_5, as monocalcium phosphate per pot, and the other with a high basal dressing consisting of 100 mg. N and 100 mg. P_2O_5 per pot. In both series the effect of potash applied as potassium sulphate at increasing rates from 0 – 100 and 0 – 250 mg. K_2O per pot was studied.

In order to improve aeration each pot was fitted with an unsymmetrical glass U-tube of 5-mm. diameter with 5 holes pierced in the bend.

Results

Before discussing the results it may be mentioned that the figures given in the tables represent the mean of three experimental values.

In figure 6 and table I the values obtained with increasing applications of potash in conjunction with a low basal dressing are compared with those

obtained with varying amounts of potash in conjunction with a high basal dressing. The leaves used in both cases were from 25-day-old plants.

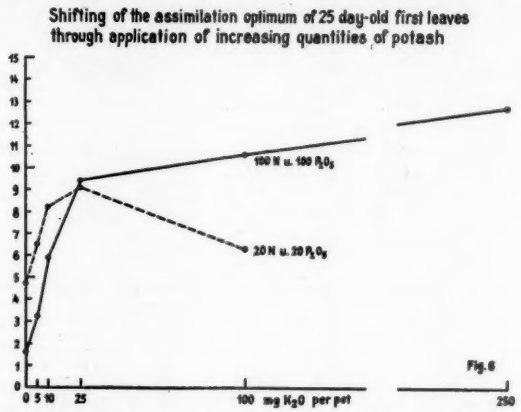

Shifting of the assimilation optimum of 25 day-old first leaves through application of increasing quantities of potash
100 N u. 100 P_2O_5
20 N u. 20 P_2O_5
0 5 10 25 100 mg K_2O per pot
250
Fig. 6

FIG. 6. The shifting of the assimilation optimum of 25-day-old first leaves through application of increasing quantities of potash.

TABLE I

CARBON DIOXIDE ASSIMILATION PER HOUR PER 100 SQ. CM. OF LEAF SURFACE

K_2O PER POT	20 MG. N AND 20 MG. P_2O_5	100 MG. N AND 100 MG. P_2O_5
mg.	*mg.*	*mg.*
0	4.7	1.6
5	6.5	3.2
10	8.2	5.9
25	9.1	9.4
100	6.3	10.6
250	.....	12.7

As these figures show, the assimilation reached its maximum in the plants receiving 25 mg. per pot in conjunction with a low basal dressing, while in the series receiving a high basal dressing the maximum was not reached until the potash application was increased to 250 mg. K_2O per pot; in the latter case the ratio $N:K_2O$ in the fertilizer was 1:2.5. In other words when heavy dressings of nitrogen and phosphoric acid are used, the potash application must be increased considerably in order to raise assimilation to its maximum.

The increase in the assimilation values with increasing applications of potash is not confined to one stage of growth only but may be observed in plants of all ages, as the following table shows.

TABLE II

ASSIMILATION BY WHEAT LEAVES OF DIFFERENT AGES (FIRST LEAVES) WITH INCREASING APPLICATION OF POTASH AND 100 MG. NITROGEN

K_2O PER POT	13 DAYS	18 DAYS	25 DAYS	30 DAYS
mg.	*mg.*	*mg.*	*mg.*	*mg.*
0	3.4	2.7	1.2	.....
5	4.3	6.7	3.0	0.0
10	6.5	11.1	6.6	3.1
25	8.9	12.9	9.2	6.3
100	(6.5)	13.9	11.4	6.8
250	8.6	14.9	12.5	7.6

At all stages of growth at which the plants were examined, *viz.*, after 13, 18, 25, and 30 days, the increase in assimilation with increasing potash applications was rapid up to an application of 25 mg. K_2O per pot, after which the rate of increase tended to slow down gradually.

The curves given in figure 7 show the relationship between assimilation and the age of the plants.

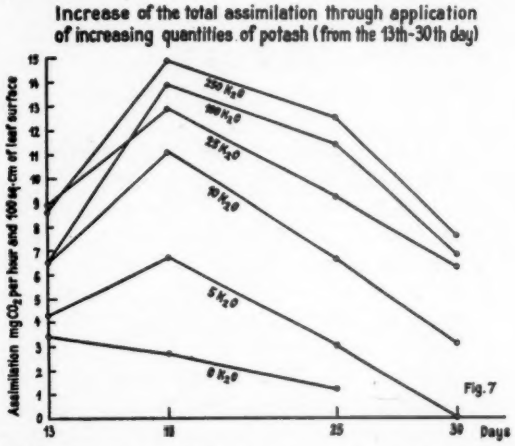


FIG. 7. Assimilation rates at different ages of the plants with respect to varying amounts of potash.

It is seen that apart from the "No Potash" series the assimilation values corresponding to the different potash treatments increase from the 13th to the 18th day and then commence to fall; this falling off of assimilation with increasing age is however much more rapid in the case of the plants receiving the low potash dressings than in that of the "High Potash" plants. This is in accordance with the fact, often observed under practical condi-

tions, that the leaves of potash deficient plants cease to function normally much earlier than those of plants sufficiently supplied with this nutrient,—RICHARDS and TEMPLEMAN (**20**); SINGH and LAL (**26**); ECKSTEIN, BRUNO and TURRENTINE (**5**).

Figure 7 also shows the total assimilation of carbon dioxide by the leaves from each treatment during the growing period. This is represented by the area enclosed by the assimilation curve for the particular manuring. This area is greater the heavier the potash application to which the curve corresponds and reaches a maximum at 250 mg. K_2O per pot, which means that not only the intensity of assimilation but also that the actual production of vegetable matter must be greater in grasses receiving adequate amounts of potash than in potash deficient grasses.

The intensity of illumination during the growth of the experimental plants was not quite sufficient for optimal development, whereas, during the actual assimilation determinations, light conditions were optimal. For this reason one cannot say with certainty, that the differences in the assimilation of carbon dioxide by the differently treated plants are really so marked during the growth of the actual plants as the experimental values would lead one to believe. At all events it is remarkable that in spite of the inadequacy of illumination during growth, the leaves from the plants receiving liberal amounts of potash proved the most efficient in respect to assimilation when light conditions were improved. In this connection reference may be made to an observation of RUSSELL'S (**21**) that the response to liberal potash manuring is particularly marked in seasons lacking in sunshine. This effect of potash manuring has also been studied experimentally by SCHARRER and SCHROPP (**22**) and by FRANK (**7**). The ordinary type of experiment, in which accurate control of all growth factors is not possible, is hardly suited to such work. We hope, however, by means of the apparatus described above to contribute to the solution of this important problem.

The results of the experiments at present under discussion confirm and amplify observations made by other workers (**18**) that the efficiency of the assimilation mechanism of the plant is increased by increasing potash applications, independently of all other factors.

As has been stressed in the introduction to this paper, the different physiological processes are interdependent. On this account we supplemented our assimilation studies by investigations on the factors influencing transpiration, protein formation and chlorophyll content.

In order to facilitate the comparison of the effects of the different potash applications, the values obtained in each case have been expressed as percentages of those obtained from an application of 100 mg. K_2O in figure 8

and table III, in which the values represent those obtained with leaves from 25-day-old plants.

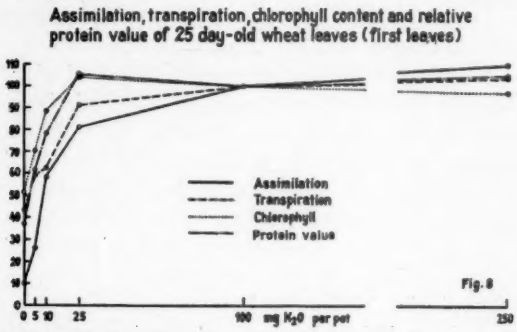

Assimilation, transpiration, chlorophyll content and relative protein value of 25 day-old wheat leaves (first leaves)
Assimilation
Transpiration
Chlorophyll
Protein value
Fig. 8
0 5 10 25 100 mg K_2O per pot 250

FIG. 8. Assimilation, transpiration, chlorophyll content and relative protein value of the first leaves of 25-day-old wheat plants.

TABLE III

QUARTZ SAND WITH HIGH BASAL MANURING (100 MG. N + 100 MG. P_2O_5 PER POT)

K_2O PER POT	ASSIMILATION CO_2 PER HOUR PER 100 SQ. CM. LEAF SURFACE	TRANSPIRATION H_2O PER HOUR PER 100 SQ. CM. LEAF SURFACE	CHLOROPHYLL* CONTENT K PER 100 SQ. CM. LEAF SURFACE	RELATIVE PROTEIN VALUE†
mg.	*mg.*	*mg.*		
0	1.2	355	1.01	1.7
5	3.0	445	1.38	2.7
10	6.6	465	1.74	3.5
25	9.2	685	2.05	4.6
100	11.4	750	1.97	4.4
250	12.5	780	1.91	4.6

* K = photometric extinction coefficient.
† Ratio of protein N to soluble N.

In our investigations of the effect of potash manuring on transpiration we found that transpiration increases with increasing applications of potash and reaches its maximum at 250 mg. K_2O. The transpiration curve is therefore similar in type to the assimilation curve, as is evident from figure 8.

The manner in which these two values are connected has not yet been definitely ascertained. STÅLFELT (30) found that when the diameters of the stomata lie between 2 and 4 μ both transpiration and assimilation are dependent on the diameter of the stomata. As a result of similar investigations GASSNER and GOEZE (9) arrived at the same conclusion. In connection with the assimilation studies the diameters of the stomata of wheat leaves were determined directly by means of the microscope after the method of STÅLFELT (29). The leaves used for this purpose were, as in the assimilation studies, exposed in the assimilation chamber to the light from

the two 1000-watt lamps for 20 minutes and then examined under the microscope. Measurements were taken of 10 stomata on the upper and lower surfaces of the leaves and it was found that, independent of manurial treatment, the diameters of the stomata on the upper surfaces of the leaves lay between 2 and 3 μ and of those on the under surfaces between 1–2 μ. This would indicate that the diameter of the stomata was not influenced by potash applications increasing from 0 – 250 mg. K_2O, although the values obtained correspond to the limits within which, according to STÅLFELT (**30**), both assimilation and transpiration show a rising tendency. Measurements carried out on leaves taken directly from the experimental plants yielded similar negative results. Working with mustard plants growing in water cultures MÜLLER and LARSEN (**18**) were also unable to establish any correlation between the diameter of the stomata and the changes in the production of organic matter in the plant due to potash and nitrogen deficiencies.

It is intended however to repeat these investigations on a greater number of plants with a view to verifying the foregoing results. Furthermore the mutual relationship existing between diameter of the stomata, water economy and the effect of potash is a question which has still to be investigated thoroughly. The results obtained in the investigations on the effect of potash on the water economy of plants, carried out up till the present, are rather conflicting. GASSNER and GOEZE (**9**) as well as SCHMALFUSS (**25**) emphasize the fact, that in most of these experiments the intake by and loss of water from the plant have not been studied separately, so that the observed loss in weight represents only the resultant effect of both processes. The contradictory nature of the results obtained may at least in part be attributed to the variety of methods used by these workers. On the other hand the particular combination of nutrient salts used is also of importance; for instance working with water cultures HANSTEEN-CRANNER (**12**) claims to have observed an increase, and SNOW (**28**) a decrease, of transpiration due to potash deficiency. The importance not only of the absolute amounts of K_2O and N used but also of the ratio in which they are supplied to the plant, has been already stressed sufficiently in the discussion of the influence of potash manuring on assimilation.

In our experiments transpiration was found to proceed parallel to assimilation. In plants receiving sufficient nitrogen both increase with increasing applications of potash, while in nitrogen deficient plants both tend to be more or less markedly depressed by heavy potash manuring.

These results obtained with cut leaves correspond to those obtained by SCHIECK (**23**), GASSNER and GOEZE (**9**) and ALTEN and GOEZE (**3**), which showed that any improvement in conditions with respect to nutrition produced a corresponding increase in transpiration; they do not however permit of any conclusions being drawn from them in regard to the influence of

nutrition on the water economy of the plant. It remains for further investigations to establish a relationship between the above results and the physiological functioning of the stomata on the one hand and the results of practical experience in respect to the effect of manuring on the consumption of water by agricultural crops on the other.

The same material, as used in the assimilation and transpiration studies, was also utilized for the protein studies. The results obtained are given in table III and figure 8.

In these investigations the ratio protein nitrogen/soluble nitrogen, the so-called "relative protein value," PAECH (19), was adopted as a criterion of protein formation. This value expresses the momentary state of equilibrium in protein metabolism and therefore the capability of the leaves to synthesize proteins. A low relative protein value indicates a high content of soluble nitrogen and in such cases the efficiency of the leaf for building up protein is impaired, so that the breaking down of the proteins tends to predominate. This can occur, for example, in older leaves as shown by MOTHES (16, 17) and SMIRNOW (27).

As may be seen from figure 8 the relative protein value of grasses increases with increasing potash applications. In contrast to the assimilation values the protein value reaches its maximum in plants receiving 25 mg. K_2O and then remains constant even with the highest dose of potash fertilizer. In grasses suffering from a marked deficiency of potash, the functional disturbances producing low assimilation values are evidently also accompanied by disturbances of the processes involved in protein formation.

The relationship between assimilation and protein metabolism will be discussed later in connection with their mutual dependence on the age of the plant. As the experimental values (figure 8) indicate the chlorophyll content at first increases with increasing applications of potash, similar to assimilation, but in contrast to the latter reaches its maximum at 25 mg. K_2O and falls slightly when the potash application is increased beyond this limit.

In agricultural practice special value is often attached to a high chlorophyll content of the leaf and attempts have been made to correlate chlorophyll content with the probable height of yield. That a relationship exists between chlorophyll content and assimilation has been demonstrated by EMERSON (6) and others and the results obtained by GASSNER and GOEZE (9) point in the same direction. The latter authors have pointed out, however, that assimilation may be very weak in the presence of a high chlorophyll content, while WILLSTÄTTER and STOLL (31) have produced evidence to show that a direct proportionality need not exist between chlorophyll content and assimilation. In this connection see also MÜLLER and LARSEN (18). This is also indicated by the experimental values given in figure 8,

which show that the leaves from plants receiving 250 mg. K_2O had a comparatively low chlorophyll content. From this it would seem that high potash applications tended to depress the chlorophyll content of the leaf. Similar observations have been made by MAIWALD (15) on potato plants. On the other hand our results also show that a depression of the chlorophyll content does not mean that the functioning of the leaf, in this case the synthesis of organic matter, is impaired.

Conversely it can also happen that grasses in poor condition may exhibit a high chlorophyll content. A very typical case is presented by the results obtained with the second leaves, as can be seen from figure 9 and table IV.

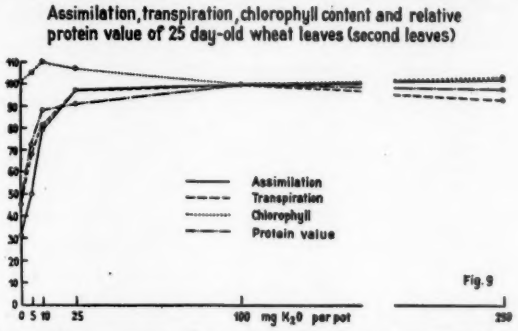

Assimilation, transpiration, chlorophyll content and relative protein value of 25 day-old wheat leaves (second leaves)
Assimilation
Transpiration
Chlorophyll
Protein value
Fig. 9
0 5 10 25 100 mg K2O per pot 250

FIG 9. Assimilation, transpiration, chlorophyll content and relative protein value of the second leaves of 25-day-old wheat plants.

TABLE IV

ASSIMILATION, TRANSPIRATION, CHLOROPHYLL CONTENT AND RELATIVE PROTEIN VALUE OF THE SECOND LEAVES OF 25-DAY-OLD WHEAT PLANTS

K_2O PER POT	ASSIMILATION OF CO_2 PER HOUR PER 100 SQ. CM. LEAF SURFACE	TRANSPIRATION H_2O PER HOUR PER 100 SQ. CM. LEAF SURFACE	CHLOROPHYLL* CONTENT K PER 100 SQ. CM. LEAF SURFACE	RELATIVE PROTEIN VALUE†
mg.	*mg.*	*mg.*		
0	3.7	260	2.16	1.9
5	6.0	395	2.25	3.1
10	9.6	470	2.36	3.8
25	11.8	565	2.28	3.9
100	12.1	580	2.14	4.3
250	12.4	540	2.20	4.4

* K = photometric extinction coefficient.
† Ratio of protein N to soluble N.

In this case the highest chlorophyll content corresponded to the lower potash applications, while the same leaves gave very low assimilation values. In spite of the high chlorophyll content, which imparted a dark green color to the leaves, the functioning of the latter was impaired. Neither a dark green color nor a high chlorophyll content of the leaf can therefore be

accepted without reserve as being a reliable indication of a high production of organic matter.

The transpiration and relative protein values display the same characteristics as in the case of the first leaves, that is they increase parallel to the assimilation values with increasing applications of potash. In contrast to the chlorophyll values they also give evidence of functional disturbances in the case of plants receiving inadequate amounts of potash.

The extent to which assimilation and protein values are dependent on the age of the leaves will be dealt with briefly. Curves showing this relationship for plants receiving 250 mg. K_2O are given in figure 10, the different values for both assimilation and protein content being expressed as percentages of the maxima.

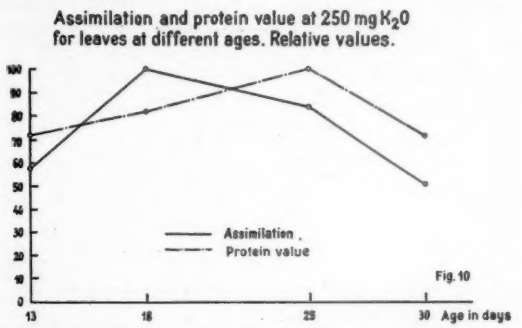


FIG. 10. Assimilation and protein values for leaves at different ages from plants supplied 250 mg. K_2O.

Whereas assimilation reaches its optimum in 18 days, the optimum for the relative protein values is reached after 25 days. Prior to this stage assimilation has already begun to fall off, *i.e.*, the functioning of the assimilation apparatus is impaired; nevertheless the protein value continues to rise until the 25th day and does not start to fall until the assimilation value has sunk to 85 per cent. of the optimum.

DESSAUERSTRASSE 28/29
BERLIN, GERMANY

LITERATURE CITED

1. ALTEN, F., and GOEZE, G. Untersuchungen über die Veränderungen der anatomischen Struktur des Halmes and ihre Beeinflussung durch Kalidüngung bei Hafer und Gerste. Ernäh. d. Pflanze **31**: 181–193. 1935.
2. ————, ————. Der Einfluss der Düngung auf den Ertrag und die Güte der Flachsfaser. Ernäh. d. Pflanze **32**: 1–14. 1936.

3. ————, ————. Versuche über die Wirkung des Kaliums auf die Kohlensäureaufnahme von Weizenblättern. Ernäh. d. Pflanze **33**: 21–28. 1937.
4. ECKSTEIN, O. Arbeiten über Kalidüngung **II**: 290–300. Berlin, 1935.
5. ————, BRUNO, A., and TURRENTINE, J. W. Potash deficiency symptoms. Berlin, 1937.
6. EMERSON, R. The relation between maximum rate of photosynthesis and concentration of chlorophyll. Jour. Gen. Physiol. **12**: 609–622. 1929.
7. FRANK, A. Die Beteiligung des Kaliums an der Stofferzeugung der höheren Pflanzen. Teil II. Untersuchungen über das Verhalten einiger Kulturpflanzen bei verschieden starker Belichtung und Kaliumversorgung. Boden. und Pflanzenern. **1**: 133–168. 1936.
8. GASSNER, G., and GOEZE, G. Über den Einfluss der Kaliernährung auf die Assimilationsgrösse von Weizenblättern. Ber. d. Bot. Ges. **50 a**: 412–482. 1932.
9. ————, ————. Assimilationsverhalten, Chlorophyllgehalt und Transpirationsgrösse von Getreideblättern mit besonderer Berücksichtigung der Kalium- und Stickstoffernährung. Zeitschr. Bot. **27**: 257–340. 1934.
10. ————, and HASSEBRAUK, K. Untersuchungen über die Beziehungen zwischen Mineralsalzernährung und Verhalten der Getreidepflanzen gegen Rost. Phytopathol. Zeitschr. **3**: 535–617. 1931.
11. GREGORY, F. C., and RICHARDS, F. J. Physiological Studies in Plant Nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**: 119–161. 1929.
12. HANSTEEN-CRANNER, B. Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III. Beiträge zur Biochemie und Physiologie der Zellwand lebender Pflanzen. Jahrb. wiss. Bot., **53**: 536. 1914.
13. JAENICHEN, H. Untersuchungen über die Wirkung des Kaliums auf den Stickstoffhaushalt etiolierter und grüner Keimpflanzen. Angew. Bot. **17**: 374–445. 1935.
14. KEYSSNER, E., and TAUBOCK, K. Handbuch d. Pflanzenanalyse. Bd. 4. Berlin, 1933.
15. MAIWALD, K. Wirkung höher Nährstoffgaben auf den Assimilationsapparat. Versuch einer Klärung der physiologischen Zusammenhänge zwischen Farbe, Chlorophyllgehalt und Zusammensetzung des Kartoffellaubes unter dem Einfluss insbesondere verschiedener Formen der Kaligabe. Angew. Bot. **5**: 33–48. 1923.

16. MOTHES, K. Ein Beitrag zur Kenntnis des N-Stoffwechsels höherer Pflanzen (unter Ausschluss des Keimlingsstadiums und unter besonderer Berücksichtigung der Säureamide). Planta **1**: 472–552. 1926.
17. ————. Zur Kenntnis des N-Stoffwechsels höherer Pflanzen, 3 Beitrag. (unter besonderer Berücksichtigung des Blattalters und des Wasserhaushaltes). Planta **12**: 686–731. 1931.
18. MÜLLER, D., and LARSEN, P. Analyse der Stoffproduktion bei Stickstoff- und Kalimangel. Planta **23**: 501–517. 1935.
19. PAECH, K. Über die Regulation des Eiweissumsatzes und über den Zustand der proteolytischen Ferment in den Pflanzen. Planta **24**: 78–129. 1935.
20. RICHARDS, F. J., and TEMPLEMAN, W. G. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. **50**: 367–402. 1936.
21. RUSSEL, J. 85 Jahre Düngungversuche in Rothamsted, England. Kali und Landwirtschaft: 71–92. Berlin, 1928.
22. SCHARRER, K., and SCHROPP, W. Über die Wirkung des Kalium-ions bei mangelnder Lichtversorgung. Zeit. Pflanzenernähr. Düng. u. Bodenk., T. A. **35**: 185–193. 1934.
23. SCHIECK, W. Einfluss von Kalium auf Assimilation und Transpiration von Sommergerste. Arch. Pflanzenbau, A. **6**: 412–464. 1933.
24. SCHMALFUSS, K. Untersuchungen über den Eiweissstoffwechsel von Kalimangelpflanzen. Phytopathol. Zeitschr. **5**: 207–249. 1933.
25. ————. Das Kalium. Eine Studie zum Kationenproblem im Stoffwechsel und bei der Ernährung der Pflanzen. Naturw. u. Landw., Heft 19. Freising-München. 1936.
26. SINGH, B. N., and LAL, K. N. Investigation on the effect of age on assimilation of leaves. Ann. Bot. **49**: 291–307. 1935.
27. SMIRNOW, J. Über die biochemischen Eigentümlichkeiten des Alterns der Laubblätter. Planta **6**: 687–766. 1928.
28. SNOW, A. G., JR. Transpiration as modified by potassium. Plant Physiol. **11**: 583–594. 1936.
29. STÅLFELT, M. G. Handbuch d. biol. Arbeitsmethode, **XI**: 167. 1929.
30. ————. Die Transpiration und Kohlensäureassimilation bei Blättern und Stroh des Hafers. Angew. Bot. **17**: 157–190. 1935.
31. WILLSTÄTTER, R., and STOLL, A. Untersuchungen über die Assimilation der Kohlensäure. Berlin, J. Springer, 1918.

ESTIMATION OF PROTOPLASMIC PERMEABILITY FROM PLASMOLYTIC TESTS

G. W. Scarth

Cell permeability as estimated by plasmolytic and other methods, while it may not go far to explain the problems of active absorption and translocation of materials, is none the less a highly significant factor in various physiological phenomena. Recent investigations by co-workers of the writer have shown, for example, that increase of cell permeability plays an important part in some quite unsuspected connections, *viz.*, in frost resistance (Levitt and Scarth, 1), drought resistance (Whiteside, unpublished results) and infection by rusts (Thatcher, 3). It would seem therefore that estimation of permeability might well be more commonly applied to the study of physiological problems, and the purpose of the present paper is to give some hints on technique and calculation which may encourage and aid this type of research. Its more particular purpose is to give formulae for the estimation of what Collander calls protoplasmic permeability, *i.e.*, permeability per unit area of protoplast. Commonly the immediate research problem in hand requires only a knowledge of relative permeability, but as will be demonstrated, little extra labor is required to furnish an "absolute" value which is independent of the size, shape and osmotic pressure of the particular cells to which it refers. Accumulation of data in this form would gradually build up a body of knowledge of comparative cell physiology.

Principles of permeability estimation

It may be necessary at the outset to defend the validity of plasmolytic methods. Under such treatment do cells behave as passive osmotic machines? Some tissues in fact do not. Shock effects appear in the form of "false plasmolysis" or "vacuolar contraction"—phenomena not governed apparently by simple osmotic relations. Unless recovery is rapid such tissues are unsuited to the technique—but they are the exception not the rule. More commonly we find occasional cells affected near the margin of a preparation which otherwise behaves normally. The abnormal cells may refuse to deplasmolyze with the others and often show a different color when stained with neutral red. Such cells have to be ignored. The fact remains, however, that as a rule cells do respond to osmotic changes in the environment in the way which is to be expected of them as sacs which are semipermeable to certain solutions and not to others.

Another question asked is: Do the results obtained by plasmolysis apply to cells under more normal conditions? With certain reservations, the writer believes that they do apply as regards "passive permeability." Thus,

differences in water permeability as displayed in plasmolysis tests are paralleled by the rate at which water passes out of cells on freezing. Solute permeability also is the same whether measured with or without plasmolysis. Also the osmotic value of the cell sap is usually found to be unchanged at the end of a permeability test. The reservations referred to are two mechanical effects which may temporarily produce abnormal permeability. One is too violent separation of the protoplast from the wall when there is a strong tendency to adhesion as shown by irregular plasmolysis shapes, and the other is too rapid deplasmolysis especially when the protoplast is highly viscous. Both effects can be avoided, the first by preliminary gradual plasmolysis and the second by slowing down deplasmolysis.

The far-reaching results of many prominent investigators are proof of the value of plasmolytic methods. No other technique has contributed so much to our knowledge of osmotic and permeability relations—witness the contributions of OVERTON, DE VRIES, FITTING, and the modern schools of HÖFLER and COLLANDER, to mention only a few. No other technique is so generally applicable or so speedy. Of course it must be learned and must always be used with judgment. It must be admitted, however, that quantitative estimates which take account of all the factors affecting the results are few indeed. An analysis of these factors is therefore our next step.

All plasmolytic methods determine permeability from the change over a known time in the volume of the protoplast. The volume change is either measured directly or estimated from osmotic change. Convenience usually decides that an increase rather than a decrease of volume is used. Hence the cells are first plasmolyzed and then allowed to deplasmolyze. The rate of volume change at any moment $\frac{dt}{dv}$ is expressed by the following general formula

$$\frac{dv}{dt} = Pcs \quad \text{.....(1)},$$

where P = the coefficient of permeability of the protoplasm,
c = the difference of effective osmotic pressure or concentration between the inside and outside of the protoplast, and
s = the area of the surface of the protoplast.

Regarding the factor P we might expect *a priori* that it would be affected by change in the thickness of the protoplasmic layer during plasmolysis and deplasmolysis. It has been shown, however, that *water* permeability is unaffected by the degree of plasmolysis and, although in rapid deplasmolysis there is a temporary increase of permeability, this depends on the *rate* of expansion not the *stage* of deplasmolysis (LEVITT, SCARTH and GIBBS, 2). Thus while the value obtained by deplasmolysis may sometimes be too high it ought to remain constant as long as the rate of expansion is constant. In

tests for *solute* permeability deplasmolysis is very gradual and does not usually affect permeability. The constancy of P during deplasmolysis in urea solution has been proved for cells of leaf sheath of wheat by FRASER in this laboratory (unpublished results). It was found that with long narrow cells the length of the protoplast was a linear function of the time, which, as we shall see later, demands that P do not vary. We shall therefore regard P as remaining constant during the test, while c and s are variables.

The factor c in our equation should strictly be the *gradient* of concentration or pressure, but inasmuch as in the case of a cell the thickness of the effective membrane is unknown and is moreover apparently unaltered by change in thickness of the protoplasmic layer as a whole (since P is unaltered) it is customary to regard the driving force as simply a function of a *difference* of concentration or pressure inside and outside the protoplast. Its value when water alone is able to pass through the protoplasm is evidently the difference between the total internal and external osmotic pressures. The inside osmotic pressure, or concentration, decreases as the volume of the protoplast increases; the external pressure remains unchanged.

In the presence of a penetrating solution the osmotic relations are more complex but the net result is the same. To elucidate we shall designate the component pressures by symbols.

Let o = the partial pressure of sap solutes,

c_1 = the partial pressure of penetrating solute inside,

C = the partial pressure of penetrating solute outside, and

A = the partial pressure of non-penetrating solute outside.

No other components can exist, and A is often absent. When water is being drawn into the deplasmolyzing protoplast the total osmotic pressure inside must exceed the total pressure outside to bring this about. Let the excess pressure inside = P. Then

$$o + c_1 - p = C + A$$

or

$$o - A = C - c_1 + p$$

Now $C - c_1$ is the pressure responsible for the entry of *solute* and p, by definition, that responsible for the entry of *water*. Therefore the sum of these is the total pressure causing the *solution* to enter, and this is seen to equal $o - A$, which is the same as when water alone can pass.

Thus c in equation (1) may be replaced by $o - A$ whether P stands for water permeability or "solution permeability," if we may use such a term, hence

$$\frac{dv}{dt} = P\,(o - A)\,s$$

It is to be noted that this formula gives only the *volume* of solution taken up. Before the rate of entry of *solute* can be determined it is necessary to

know either the *concentration* in which the solution enters or the partial pressure of the penetrating solute in the sap. The formula, however, is a first step in the estimation of solute permeability and further steps will be dealt with later.

Variation of the factor s is one of the complications inherent in the plasmolytic method. Important though surface area is, most authors have neglected it in the past. One reason for this neglect has been their uncertainty as to how much of the surface of the protoplasts is freely available in sections of tissue. If there is any blanketing as regards diffusion by contiguous cells this effect ought to be at a maximum in the middle of a section and be manifest by a lag in deplasmolysis in this region as compared with the marginal cells. With thin sections of parenchymatous tissue no such effect appears, however (THATCHER, 3). In such preparations evidently the whole surface of every protoplast is more or less equally utilized in osmosis —at least until deplasmolysis is almost complete—and the permeability of its own protoplasm is so far the limiting factor for each cell that other interference is negligible. This condition cannot hold so completely in epidermal preparations, but for the great bulk of plant tissue its attainment is probably near enough, provided the section is for the most part only one cell thick.

The interrelationship of the factors v, c, and s has now to be considered because equation (1) is insoluble unless c and s (which are variables) can be expressed in terms of v. The relation of c to v always remains constant. The value of c, as we have seen, equals $o - A$. A is constant and o varies as $\frac{1}{v-x}$ where x is the "non-solvent space." Any change in x which may be produced by change in volume is a quite negligible one in its bound-water fraction. Very commonly x itself can be neglected, but in cells rich in colloid, as in embryonic tissues and those adapted to withstand extreme drought or frost, it may be a large part of v.

The relation of s to v depends on the shape of the cell cavity combined with the ability of the protoplast when it leaves the wall to be molded by surface tension. As a rule a minimal area form is not assumed during the process of plasmolysis but is reached either on standing in the plasmolyte, or at least after a slight degree of subsequent deplasmolysis. This is one of the reasons for preferring the deplasmolysis phase of volume change for measurement of permeability.

As long as the protoplast retains the same geometric form, s can be expressed as a function of v. With isodiametric cells the spherical form is assumed and preserved up to a certain point, which is usually considerably short of complete deplasmolysis. For a sphere $s = (4\pi)^{1/3} \cdot (3v)^{2/3}$. The commonest shape of cell in plant tissues, however, has one axis longer than the others: it is cylindric or cylindro-prismatic and here the protoplast keeps its

form until the cavity is nearly filled. The form in question is approximately that of a cylinder with hemispherical ends in which

$$s = \frac{2v}{r} + \frac{4}{3}\pi r^2,$$

r being half the diameter of the cell and therefore constant. For purpose of precise calculation it is necessary to regard the upper limit of volume in which either of the above geometric forms is preserved rather than the full volume of the cell, as the standard to which we refer as "incipient plasmolysis" or "end plasmolysis" as the case may be. With this limitation s may be expressed as a steady function of v during plasmolysis of the great majority of plant cells.

Having reached the conclusion that P does not change during a properly conducted deplasmolysis test and that the variables c and s can be kept as constant functions of v we are left with the relation of v to t, which must be learned from experiment. If only relative values of v and t are given in the equation, the constant P will stand for relative or "protoplast permeability"; if their absolute values are used, P represents "protoplasmic permeability," a much more definite property. Our next step therefore is to solve the basic equation and derive formulae which are convenient for quantitative experiments with the principal types of cell.

Formulae for cylindric cells

Derivation of a general formula.—Let us consider equation (1) as it applies to cylindrical cells of all kinds.

Let V = volume of the protoplast at "incipient plasmolysis,"
O = osmotic value at volume V,
L = full length of the cell,
l = length of the protoplast at volume v,
x = non-solvent space,
y = v − x, and
Y = V − x.

For the sake of simplicity we exclude here the presence of a non-penetrating solution outside, a condition dealt with later.

We have seen that s and c are constant functions of v and hence of y namely,

$$s = \frac{2(v + \frac{2}{3}\pi r^3)}{r} = \frac{2(y + x + \frac{2}{3}\pi r^3)}{r}$$

and

$$c = \frac{Y}{y}O; \text{ also } dy = dv.$$

Substituting in equation (1),

$$\frac{dy}{dt} = \frac{2POY}{r}\left(1 + \frac{x + \frac{2}{3}\pi r^3}{y}\right)$$

$$= a + \frac{b}{y}, \text{ where } a = \frac{2POY}{r},$$

$$\text{and} \quad b = \frac{2POY}{r}(x + \tfrac{2}{3}\pi r^3).$$

Separating the variables and integrating,

$$\int \frac{y}{ay+b}dy = \int dt$$

$$\frac{1}{a^2}\left[b + ay - b \log_e (b + ay)\right] = t + C.$$

To evaluate the constant of integration C, let $v_o = v$ when $t = o$ whence

$$C = \frac{b + ay_o}{a^2} - \frac{b}{a^2} \log_e (b + ay_o).$$

Substituting for C in the previous equation,

$$\frac{b}{a^2} \log_e \frac{b + ay_o}{b + ay} + \frac{b + ay}{a^2} = t + \frac{b + ay_o}{a^2}$$

Restoring the equivalents for a and b and substituting $v - x$ for y this becomes

$$P = \frac{r}{2tO}\left(\frac{v - v_o}{V - x} - \frac{x + \frac{2}{3}\pi r^3}{V - x} \cdot \log_e \frac{\frac{2}{3}\pi r^3 + v}{\frac{2}{3}\pi r^3 + v_o}\right) \quad \text{.....(2).}$$

This equation may be further modified according to the data which the experimental technique provides. Two methods are in use, the "end plasmolysis" and the "plasmometric," though to estimate absolute permeability when non-solvent space is present the former method requires almost as much measurement as the latter.

With the *"end plasmolysis"* or *"deplasmolysis time"* method the initial and final volumes of the protoplast are fixed. The initial is the minimum volume which it assumes in the plasmolyte and when non-solvent space is negligible it is simply calculated from the known concentration of the plasmolyte. When, however, non-solvent space is appreciable the minimum volume must be measured and the technique becomes a special case of the plasmometric. The final volume is that at "incipient plasmolysis," which can be estimated at leisure from the length and diameter of the whole cell or average of cells in a section.

There are certain advantages in using the plasmolyte at an osmotic value which bears a definite ratio to that of the cell at incipient plasmolysis which we call O. This is better than always using the same concentration because it induces uniform changes in volume and surface area (as long as x is constant or negligible) so that if the complications of blanketing and expansion effect are not eliminated they tend to operate equally in all tests. Besides, the procedure simplifies calculation. If the value *twice* O be chosen and if the volume of the cell in equilibrium with the plasmolyte be $v_{min.}$, then in equation (2) $x = 2\,v_{min.} - V$. In the following formulae the osmotic pressure of the plasmolyte will be taken as equal to $2 \times O$.

A formula for the deplasmolysis time method is obtained from equation (2) by putting V for v, $v_{min.}$ for v_o, and $2v_{min.} - V$ for x. It is also more convenient to express v as $(l - \frac{1}{3}d)\pi r^2$. This gives us

$$P = \frac{d}{8tO}\left[1 - \frac{2l_{min.} - L}{L - l_{min.}} \log_e \frac{L}{l_{min.}}\right] \quad \ldots\ldots (3).$$

The plasmometric technique proper differs from the deplasmolysis time method merely in that *any* two or more lengths of the protoplast are measured at known time intervals. If these lengths be l_1 and l_2 we get the following formula for the plasmometric method:

$$P = \frac{d}{8tO}\left[\frac{l_2 - l_1}{L - l_{min.}} - \frac{2l_{min.} - L}{L - l_{min.}} \cdot \log_e \frac{l_2}{l_1}\right] \quad \ldots\ldots (4).$$

The plasmometric technique is better adapted to measuring solute than water permeability and is the only legitimate one for solutes which show an extremely slow rate of penetration.

SIMPLIFICATION WHEN X IS NEGLIGIBLE.—As non-solvent space is so commonly negligible in plant cells we shall consider particularly how equation (2) may be simplified for this case and for use with different experimental techniques.

Deplasmolysis time method.—When $x = 0$ and the cells are plasmolyzed in a solution of $2 \times O$, v_o (*i.e.*, $v_{min.}$) $= \frac{1}{2}V$. Substituting these values and also V for v in equation (2) we get

$$P = \frac{d}{8tO}\left(1 - \frac{2d}{3(L - \frac{1}{3}d)} \log_e \frac{2L}{L + \frac{1}{3}d}\right) \quad \ldots\ldots (5).$$

Values of the last term in the above equation for various $\frac{L}{d}$ ratios are given in the following table.

TABLE I

$\frac{L}{d}$	$\left[1 - \frac{2d}{3(L - \frac{1}{3}d)} \log_e \frac{2L}{L + \frac{1}{3}d}\right]$	$\frac{3(L - \frac{1}{3}d)}{3L + \frac{1}{3}d}$
2	0.78	0.79
3	0.85	0.86
4	0.89	0.89
5	0.91	0.91
6	0.92	0.92
7	0.93	0.93
8	0.94	0.94
9	0.95	0.95

Plasmometric method.—For the plasmometric method using the same symbols as before we get,

$$P = \frac{d}{4tO}\left[\frac{l_2 - l_1}{L - \frac{1}{3}d} - \frac{d}{3(L - \frac{1}{3}d)} \log_e \left(\frac{l_2}{l_1}\right)\right] \quad \ldots\ldots (6).$$

Approximations.—The experimental error is likely to be such that with cells having $\frac{L}{d} \geqq 4$ the following approximate formulae are adequate:

End-plasmolysis method,

$$P = \frac{d}{8tO} \quad \text{..... (7).}$$

Plasmometric method,

$$P = \frac{d(l_2 - l_1)}{4tOL} \quad \text{..... (8).}$$

Relative permeability.—For the same size and shape of cell the following simple formula is accurate for relative permeability as tested by end plasmolysis:

$$K = \frac{1}{tO},$$

where K is a constant factor for permeability.

FORMULAE FROM A SIMPLE EQUATION.—If the rate of volume change is constant there is no reason to use calculus because the following simple equation is equally valid:

$$P = \frac{v - v_o}{tc_m s_m} \quad \text{..... (9),}$$

where c_m and s_m are the effective pressure difference and the surface area respectively at the mean volume $\frac{v + v_o}{2}$. This condition is approached when $\frac{L}{d}$ is high. To discover the error at lower $\frac{L}{d}$ rates we compare the results with those obtained from equation (5) and table I. When $x = 0$ and the concentration of the plasmolyte is $2 \times O$, equation (9) applied to end plasmolysis becomes

$$P = \frac{3d(L - \frac{1}{3}d)}{8tO(3L + \frac{1}{3}d)} \quad \text{..... (10).}$$

Here the expression $\frac{3(L - \frac{1}{3}d)}{3L + \frac{1}{3}d}$ takes the place of the bracketed expression in equation (5). Comparing the values of these two factors as given in table I we see that even for $\frac{L}{d}$ ratios of 2 and 3 the difference is quite negligible. It is to be noted that this close agreement of the approximate formula with the exact one is obtained by taking c_m and s_m *not* as the mean pressure difference and mean surface area respectively but as the values of these two factors *at the mean volume.*

We conclude from this near agreement that where the calculus form of equation is cumbersome or insoluble the simple form may safely be employed. Some cases in point will now be dealt with.

Approximation when $\frac{L}{d}$ *and* x *are both large.*—When x is large the final term in equation (2) cannot be neglected in spite of a high $\frac{L}{d}$ ratio. Equation (9), however, applied to the end-plasmolysis method with a plasmolyte of concentration $2 \times O$ gives a usable formula:

$$P = \frac{3(L - l_{min.})d}{8tO(L + l_{min.})} \text{ approx.} \quad \ldots\ldots (11).$$

Deplasmolysis in a hypotonic non-penetrating solution.—The difficulty in estimating water permeability is the speed with which deplasmolysis takes place, which not only makes measurement of t difficult but also produces an abnormal degree of permeability. To some extent this is overcome by using a not too hypotonic solution instead of water as the deplasmolyte. As calculus treatment of this condition for cells in general is complicated we again resort to equation (9).

When the plasmolyte has a concentration $2 \times O$ and the deplasmolyte a concentration of $\frac{1}{2}O$

$$c_m = O(4/3 - \tfrac{1}{2}) = \tfrac{5}{6}O.$$

Therefore for cylindric cells in general we get from (9)

$$P = \frac{3(L - \frac{1}{3}d)d}{5tO(3L + \frac{1}{3}d)} \text{ approx.} \quad \ldots\ldots (12),$$

and for cells with a high $\frac{L}{d}$ ratio:

$$P = \frac{d}{5tO} \text{ approx.} \quad \ldots\ldots (13).$$

This latter condition can be treated easily by calculus and gives the result:

$$P = \frac{d}{2tO} \cdot \log_e 3/2 = \frac{0.405d}{2tO}$$

which is practically the same as (13).

Formulae for spherical protoplasts

Formula when x is negligible.—Substituting the respective functions of v for c and s in equation (1) we get

$$\frac{dv}{dt} = PO\frac{V}{v}(4\pi)^{1/3}(3v)^{2/3}$$

Separating the variables and integrating,

$$\int v^{1/3}dv = \int (POV(4\pi)^{1/3}3^{2/3})dt$$

whence,

$$\tfrac{3}{4}v^{4/3} = (POV(4\pi)^{1/3}(3)^{2/3})t + C.$$

Let $v = v_o$ when $t = o$, then $C = \frac{3}{4}v_o^{4/3}$

Substituting for C and transposing,

$$P = \frac{\frac{3}{4}(v^{4/3} - v_o^{4/3})}{tOV(4\pi)^{1/3}3^{2/3}} \quad \ldots\ldots (14).$$

Plasmometric method.—Let r, r_o and R be the radii at v, v_o and V respectively.
Then from (14),

$$P = \frac{r^4 - r_o^4}{4tOR^3} \quad \text{..... (15).}$$

Deplasmolysis-time method.—Here r = R and if the concentration of plasmolyte be $2 \times O$, $r^3 = \frac{1}{2}R^3$ and hence $r_o^4 = \frac{1}{2}^{4/3}R^4$. Thus equation (15) becomes:

$$P = \frac{0.15R}{tO} \quad \text{..... (16).}$$

The same result, to the second decimal place, is obtained from the simple equation (9).

FORMULA WHEN x IS APPRECIABLE.—Adapted to the end plasmolysis method and the use of a plasmolyte of concentration $2 \times O$ equation (9) may be written:

$$P = \frac{V - v_{min.}}{t\frac{4}{3}O(4\pi)^{1/3}\left[\frac{3(V + v_{min.})}{2}\right]^{2/3}}$$

$$= \frac{0.4(R - r_{min.}^3)}{tO(R + r_{min.}^3)^{2/3}} \quad \text{..... (17).}$$

SOLUTE PERMEABILITY

According to the above formulae permeability to *solutions* is estimated from increase of volume irrespective of the proportion in which water and solute enter the cell. However, the rate at which the concentration of penetrating solute increases inside the cell must be known before permeability to the *solute* can be calculated. Following the nomenclature used in the opening discussion in which the term p signifies the excess pressure inside the cell which causes the entry of water, and also assuming that the external medium consists of the penetrating solution alone, we have the following relationship at any moment of deplasmolysis:

$$p = o + c_i - C.$$

Slow penetration.—With slowly penetrating solutions it is permissible to neglect p, because, if permeability to water is great compared with that to solute, a very low value of p relative to $C - c_i$ will allow the entry of water to keep pace with that of solute. When p is negligible it follows that $C - c_i = o$ and also, since virtual osmotic equilibrium exists, the external solution must enter as such, that is, in concentration C. Hence the rate at which the solute enters is given by the product of C and $\frac{dv}{dt}$, and if P_s be used to designate solute permeability

$$C\frac{dv}{dt} = P_s os,$$

and

$$P_s = CP.$$

Thus any of the above formulae for solution permeability gives solute permeability when multiplied by the concentration of the penetrating solution outside.

For example from equation (5) we get the following for use with the end-plasmolysis method when $C = 2 \times O$:

$$P_s = \frac{d}{4t} \quad \text{..... (18).}$$

with corrections according to table I if necessary alternatively from equation (10).

$$P_s = \frac{3d(L - \frac{1}{3}d)}{4t(3L + \frac{1}{3}d)} \quad \text{..... (19).}$$

For the plasmometric method we get from equation (6),

$$P_s = \frac{C(l_2 - l_1)d}{4tO(L - \frac{1}{3}d)} \quad \text{..... (20).}$$

If $C = 2 \times O$

$$P_s = \frac{d(l_2 - l_1)}{2t(L - \frac{1}{3}d)} \quad \text{..... (21).}$$

With slowly penetrating solutes the plasmometric method obviates the need of estimating O and even of knowing the value of C. If the amount of solute that penetrates before the protoplast reaches minimum volume in the penetrating solution is negligible and if x also is negligible,

$$\frac{C}{O} = \frac{V}{v_{min.}} = \frac{L - \frac{1}{3}d}{l_{min.} - \frac{1}{3}d}$$

Substituting in equation (20) we get

$$P_s = \frac{d(l_2 - l_1)}{4t(l_{min.} - \frac{1}{3}d)} \quad \text{..... (22).}$$

Rapid penetration.—With more rapidly penetrating solutions a second approximation which takes count of p is suggested though its use has not yet been tried out.

The following simple equation is applicable to either the end plasmolysis or plasmometric method:

$$P_s = \frac{v_e c_{1e}}{t(C - c_{1m})S_m}$$

where the sub-index e indicates end values after time t and sub-index m mean values. As c_i cannot be determined directly by plasmolytic methods we substitute $C - o + p$ with a view of determining p. When applied to end plasmolysis in a solution of concentration $2 \times O$ the above equation then becomes

$$P_s = \frac{V(O + p_e)}{t(o_m - p_m)s_m} \quad \text{..... (23).}$$

where o_m is the value of o at the mean volume, namely $\frac{1}{3}O$, p_m is the mean value of p during deplasmolysis, and p_e its value at the end.

Since p is the net pressure causing water to enter, its mean value is obtained roughly from the relation of the time taken for an equal extent of deplasmolysis in the solution in question (t_{sol}) and in water (t_w), respectively. For if we neglect the fraction of the volume increase occupied by solute,

$$p_m = o_m \cdot \frac{t_w}{t_{sol}}$$

Theoretically it would seem that p, after a brief and speedy rise at the beginning of deplasmolysis, must then fall off gradually as the solute gradient $C - c_i$ declines, because its value depends on the rate that solute enters. But if p varies as $C - c_i$ it varies as $o - p$ and therefore as o. As an approximation therefore we calculate p_e from the formula $p_e = p_m \frac{O}{o_m}$. Also, since t_{sol} here equals t, $p_m = \frac{t_w}{t} o_m$. Substituting in (23) we get

$$P_s = \frac{3\left(1 + \frac{t_w}{t}\right) V}{4t\left(1 - \frac{t_w}{t}\right) s_m} \qquad (24).$$

In the case of cylindrical cells with a high $\frac{L}{d}$ ratio this becomes

$$P_s = \frac{d\left(1 + \frac{t_w}{t}\right)}{4t\left(1 - \frac{t_w}{t}\right)} \qquad (25).$$

For any solution the value of the fraction $\frac{t_w}{t}$ decides whether a formula, such as the above, or the simpler one for slowly penetrating solutions should be used.

Units of permeability value

The result for water permeability or "solution permeability" represents the rate of movement of liquid through the protoplasm per unit difference of concentration or pressure. A convenient mode of expression is microns per hour per atmosphere (since $\mu^3/\mu^2 = \mu$).

As regards solute permeability, if concentration be expressed in mols, and cell dimensions in centimeters, the amount of solute taken up per cm.^{3} will be given in *millimols*, since mols per litre (1,000 ml.) is equivalent to millimols per cm^3. The unit of concentration difference, however, is still the mol.

Technique

A brief description of the experimental techniques which suit the preceding modes of calculation may be useful. If sectioning is required the sections must be longitudinal and generally about one cell thick.

A stock solution of $CaCl_2$, or of a "balanced" mixture of NaCl and $CaCl_2$ (*e.g.* 9:1), is the best source for making up non-penetrating solutions. Salt penetrates cell walls more quickly than sugar and the salt solution keeps indefinitely in a well-stoppered bottle. This will be referred to as "standard salt solution." All experimental solutions should be preserved strictly from evaporation in covered dishes and the preparation mounted for observation only as long as is necessary.

Deplasmolysis time method

Staining.—To facilitate observation, colorless cells are vitally stained in weak neutral red (5 to 50 parts per million). Slight alkalinity of the staining medium hastens penetration of the dye, and Ca ions prevent the cell wall from staining. Tap water often supplies both requirements. Staining is usually slow and incidentally allows time for recovery from the shock of cutting.

Osmotic pressure determination.—The average osmotic value (O) of the cells at "incipient plasmolysis" (see previous discussion) is carefully determined from sections placed in a graded series of dilutions of the standard salt solution. The preparations, if they are to be used later for permeability tests, should not be left longer than necessary in the solution.

Unless non-solvent space is known to be absent, its operation must be estimated at this stage by determining the volume (V) of the protoplast at incipient plasmolysis and then plasmolyzing in $2 \times O$ salt solution and again estimating the volume (v_o). If $\frac{V}{v_o} = 2$ we proceed as in the following paragraph; if less than 2, we proceed as described later.

Permeability determination.—This method allows an average of all the cells in a section to be estimated to observe which natural or artificial coloration of the cells is almost essential.

Water permeability.—Sections are placed in a standard salt solution of concentration equal to twice O until plasmolysis is complete, say 10–15 minutes.

They are then transferred to a hypotonic solution ($\frac{1}{2}O$) and the time of immersion noted. As soon after as experience indicates to be necessary, samples are mounted for brief intervals in a drop of the same solution for microscopic observation, and the moment at which deplasmolysis is complete in 50 per cent. of the cells is judged. This is rather difficult to estimate

sometimes because of the speed of the process and the wide individual variation among the cells. As already remarked, rapid deplasmolysis commonly causes a noticeable increase of permeability. Objections also attend the use of plasmolysis-time; but after the protoplasts have rounded up, it may at least be used as a check on the effect of deplasmolysis. Large cells and non-viscous protoplasm display least abnormality both in plasmolysis and deplasmolysis. The formula for elongate cylindrical cells is $\frac{d}{5tO}$. For short cells correct from table I or use equation (12).

Solute permeability.—If the form of plasmolysis is irregular, with adhesion to the wall, the cells are first plasmolyzed in standard salt solution stronger than $2 \times O$ and returned to $2 \times O$ to give the protoplasts the required shape. Sections are then transferred to the penetrating solution having an osmotic pressure approximately equal to that of the $2 \times O$ salt solution. Tables of osmotic pressure and concentration are invaluable in this connection. If, however, plasmolysis is smooth and produces convex contours the sections may be placed directly in the penetrant solution at the concentration $2 \times O$. The time elapsing from the moment of immersion in the penetrant to completion of deplasmolysis by about half of the cells is much easier to determine with slowly penetrating solutions than with water. The formula for elongate cells is $\frac{d}{4t}$. For short cells correct from table I or use equation (9).

Plasmometric method

As this method can be applied accurately only to individual cells which have to be recognizable when the sections are remounted for later measurement, it is usually possible to select suitably long and narrow cells, which reduces the error of measurement and also simplifies calculation. Prestaining is not essential with this method. If desired, the osmotic pressure of the selected cells is determined plasmometrically, that is, by measuring the length of the protoplast in equilibrium with a known concentration either of salt solution or even of the penetrant solution itself when the rate of entry of the solute is relatively slow; but it is not necessary to know the osmotic value, either of the cell or of the penetrant, if the minimum length is measured.

To estimate permeability plasmometrically, two or more lengths (or diameters if the form is spherical) of the deplasmolyzing protoplast are carefully measured with a micrometer at known time intervals. The minimum length may be one of these. The simplest formula for cylindrical cells is (22). This method allows greater accuracy than other plasmolytic methods but only as regards the cells actually measured.

NON-SOLVENT SPACE APPRECIABLE.—After the length $l_{min.}$ (or diameter) in $2 \times O$ salt solution has been measured, the cells are deplasmolyzed in whichever medium is suitable to the object in view. If solute permeability is to be determined, it is important that the osmotic pressure of the penetrating solution be as nearly as possible the same as that of the $(2 \times O)$ solution. Disparity will be revealed by a quick change in protoplast volume when transferred to the penetrant solution.

Summary

This paper includes a discussion of the principles of estimating protoplasmic permeability plasmolytically, the derivation of comprehensive formulae for use in estimating the protoplasmic permeability of cylindrical and spherical cells, and some notes on technique.

The writer is much indebted to Dr. W. ROWLES for aid with the mathematics and for useful suggestions.

MCGILL UNIVERSITY
MONTREAL, CANADA

LITERATURE CITED

1. LEVITT, J., and SCARTH, G. W. Frost-hardening studies with living cells. II. Permeability in relation to frost resistance and the seasonal cycle. Canadian Jour. Res. C, **14**: 285–305. 1936.
2. ————, ————, and GIBBS, R. D. Water permeability of isolated protoplasts. Protoplasma **26**: 237–248. 1936.
3. THATCHER, F. Osmotic pressure and permeability relationships in the nutrition of fungus parasites. (In press.)
probably Am. Jour. Bot. 26(6): 449-458. 1939.

EFFECT OF ETHYLENE ON CERTAIN CHEMICAL CHANGES ASSOCIATED WITH THE RIPENING OF PEARS[1]

ELMER HANSEN

(WITH TWO FIGURES)

Introduction

Recent discoveries (**7, 8, 10**) have shown that ethylene occurs in small amounts as a natural metabolic product of certain fruit and other plant tissues. The fact that a gas of this nature is produced by plants is of great interest, especially in view of the fact that ethylene has been used extensively in the past as an artificial treatment to hasten ripening in various fruits. The results obtained from use of this treatment, however, have not always been uniform, and for this reason there apparently has developed a diversity of opinions as to the exact relation, if any, of ethylene to the ripening of fruit. In some cases respiration (**6**), starch hydrolysis (**14**), and other chemical changes are reported to have been affected. In other cases (**5, 16, 26**), the results obtained have indicated that there is no apparent benefit from the use of ethylene for increasing the rate of ripening in various fruits.

As an explanation for these differences, HARVEY (**15**) has suggested that the concentration of gas used in some experiments might have been too low. In other cases (**1, 27**) maturity of the fruit has evidently been a factor. It is apparent, however, that in many of these previous experiments the fact has been overlooked that during maturation and after storage, fruits naturally undergo progressive physiological changes which may greatly modify the response shown to ethylene treatment. Thus, in a former experiment with pears (**12**) it was found that the respiration of the fruit showed a definite, well-defined trend during maturation and after storage, and the response obtained with ethylene varied greatly according to the respiratory activity of the fruit at time of treatment.

In the present investigation it was considered desirable to study the effects of ethylene on certain chemical changes occurring in pears during the ripening process. By observing the comparative responses of a single variety treated at different stages of maturity and after various periods in cold storage, it was thought that any chemical changes which were definitely affected by this gas during any specific stage in the life of the fruit would become apparent. Information of this nature would be of value in determining what the effects of a natural accumulation of ethylene in fruit tissues would have upon initiation of the ripening process.

[1] Published as Technical Paper no. 285 with the approval of the Director of the Oregon Agricultural Experiment Station.

Materials and general methods

Collection and handling of samples

The fruit used in these experiments were Bartlett and Anjou pears, obtained from the Hood River and Medford (Oregon) districts. Fruit intended for treatment immediately after picking was gathered at approximately two-week intervals, beginning 5 weeks after blooming and extended several weeks beyond the regular commercial harvest season. Fruit intended for storage studies was picked on one date when the proper picking stage for the variety had been reached.

As soon as the fruit had been brought from the field or removed from storage, two uniform lots were sorted out and placed in 5-gallon glass jars. To one set of jars was added ethylene to make a concentration of 1:1000 by volume; the second set of jars was kept under constant ventilation with fresh air to prevent any accumulation of gases affecting ripening. Details of the method used to maintain comparable temperatures, humidities, and carbon dioxide and oxygen tensions in the treated and untreated jars are described in a previous publication (**12**). To compare the trend of chemical changes occurring after picking in the ethylene-treated and untreated lots, a sample of 15 pears was withdrawn for analysis at definite intervals during the course of ripening. With immature fruit, a sample was taken every 4 days during a period of 12 days. With mature and storage fruit, which ripened more rapidly, only two analyses were made: the first immediately after picking or removal from storage, and the second after a period of 8 days.

Methods of analysis

Sampling.—Samples for chemical analysis were prepared by cutting longitudinal sectors of 15 whole pears, grinding them through a food chopper, and finally weighing out duplicate 50-gm. samples of the finely ground tissue.

Extraction.—Sugars were extracted with 95 per cent. alcohol in a Soxhlet extractor for 24 to 30 hours. The alcohol was then distilled off under a 26 to 28 inch vacuum at 40° C., the water extract cleared with neutral lead acetate, de-leaded with potassium oxalate, and made to volume.

Sugar determinations.—Reducing sugars before and after inversion were determined by the method of Lane and Eynon (**19**). Inversion was carried out by the method described by Martin (**21**) and sucrose was calculated by the usual method.

Alcohol insoluble residue.—The alchohol insoluble residue was determined as the dry weight of the material remaining in the thimbles after extraction.

Starch.—This was determined on 0.5-gm. samples of the alcohol insoluble residue, finely ground with quartz sand, and digested with fresh saliva

according to the method described by LOOMIS and SHULL (**20**). The reducing values of the solutions after hydrolysis were determined by the iodometric method of SHAFFER and HARTMANN (**24**); and converted to starch by the factor 0.90.

PECTIC SUBSTANCES.—Soluble pectin and protopectin were determined by the method of CARRÉ and HAYNES (**4**) on 50-gm. samples of the fresh tissue, and are expressed as impure calcium pectate.

ACID.—Acid was determined on 50-ml. aliquots of the water solution of the alcohol extract used for sugar determinations and calculated as citric. Because of the difficulty in determining the end point with phenolphthalein indicator, the solutions were titrated electrometrically to pH 7.2 with 0.01N sodium hydroxide on a YOUDEN quinhydrone electrode.

Results

EXPERIMENTS WITH BARTLETT PEARS RIPENED BEFORE STORAGE

In order to have fruit representative of different stages of maturity, approximately 200 pears were picked at intervals throughout the later part of the growing season. The dates on which the samples were collected and the average weights of 25 fruits at time of picking are shown in table I.

TABLE I

AVERAGE WEIGHT OF FRUIT COLLECTED AT DIFFERENT PERIODS

DATE PICKED	AVERAGE WEIGHT PER FRUIT
	gm.
July 14	43.1
July 29	99.3
August 16	138.6
August 25*	150.5
September 14	160.5

* Commercial picking date.

The first picking, made on July 14, was extremely immature. The amount of ethylene produced at this stage of development was barely detectable by epinasty of potato leaves, and the tissues of the untreated fruit probably contained only traces of this gas naturally produced. Fruit picked later showed increasing evidence of ethylene production, and the tissues of the untreated fruit were probably not free from this gas, even though constant aeration was provided during the entire ripening period.

The changes in total and reducing sugars, sucrose, starch, pectic substances, alcohol insoluble residue, and acid are shown in table II, and are represented graphically, with the omission of acid and alcohol-insoluble residue, in figure 1. The percentages of increase or decrease in each material

TABLE II

EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARTLETT PEARS PICKED AT DIFFERENT STAGES OF MATURITY

No. days ripened	Total sugars		Reducing sugars		Sucrose		Soluble pectin		Insoluble protopectin		Starch		Alcohol-insoluble residue		Acid as citric	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
	1st picking—July 14															
0	4.20	4.20	4.06	4.06	0.13	0.13	0.10	0.10	0.85	0.85	1.24	1.24	6.65	6.65	0.23	0.23
4	4.86	5.85	4.62	5.32	0.23	0.50	0.10	0.31	0.84	0.36	1.29	0.76	6.32	5.41	0.17	0.17
8	5.29	6.39	5.18	5.80	0.11	0.56	0.12	0.65	0.80	0.25	1.14	0.21	5.98	5.91	0.28	0.27
12	5.39	5.72	5.18	5.26	0.20	0.44	0.09	0.21	0.85	0.18	1.01	0.14	6.13	5.42	0.19	0.17
	2nd picking—July 29															
0	6.28	6.28	5.70	5.70	0.55	0.55	0.09	0.09	0.86	0.86	1.09	1.09	4.95	4.95	0.15	0.15
4	6.54	6.82	6.08	6.43	0.44	0.38	0.08	0.57	0.90	0.42	0.85	0.60	4.70	4.45	0.18	0.19
8	6.82	7.31	6.30	6.60	0.50	0.67	0.32	0.71	0.46	0.20	0.66	0.45	4.38	3.98	0.20	0.19
12	7.72	7.60	7.15	7.00	0.55	0.57	0.51	0.38	0.13	0.10	0.39	0.28	3.54	3.59	0.23	0.23
	3rd picking—August 16															
0	6.68	6.68	6.04	6.04	0.61	0.61	0.21	0.21	0.64	0.64	0.71	0.71	4.35	4.35	0.20	0.20
4	7.29	7.59	6.54	6.75	0.71	0.80	0.26	0.39	0.68	0.52	0.85	0.68	4.05	3.76	0.20	0.21
8	7.72	7.58	6.99	6.52	0.70	1.00	0.42	0.64	0.26	0.10	0.60	0.34	3.63	3.39	0.22	0.19
	4th picking—August 25															
0	7.21	7.21	6.24	6.24	0.93	0.93	0.19	0.19	0.61	0.61	0.64	0.64	3.22	3.22	0.17	0.17
8	8.42	8.44	6.67	6.90	1.66	1.46	0.51	0.59	0.23	0.19	0.37	0.10	3.22	3.38	0.22	0.21
	5th picking—September 14															
0	8.49	8.49	6.45	6.45	1.94	1.94	0.19	0.19	0.63	0.63	0.34	0.34	2.90	2.90	0.26	0.26
8	8.87	8.97	6.49	6.56	2.26	2.29	0.65	0.68	0.19	0.15	0.21	0.19	2.42	2.41	0.15	0.21

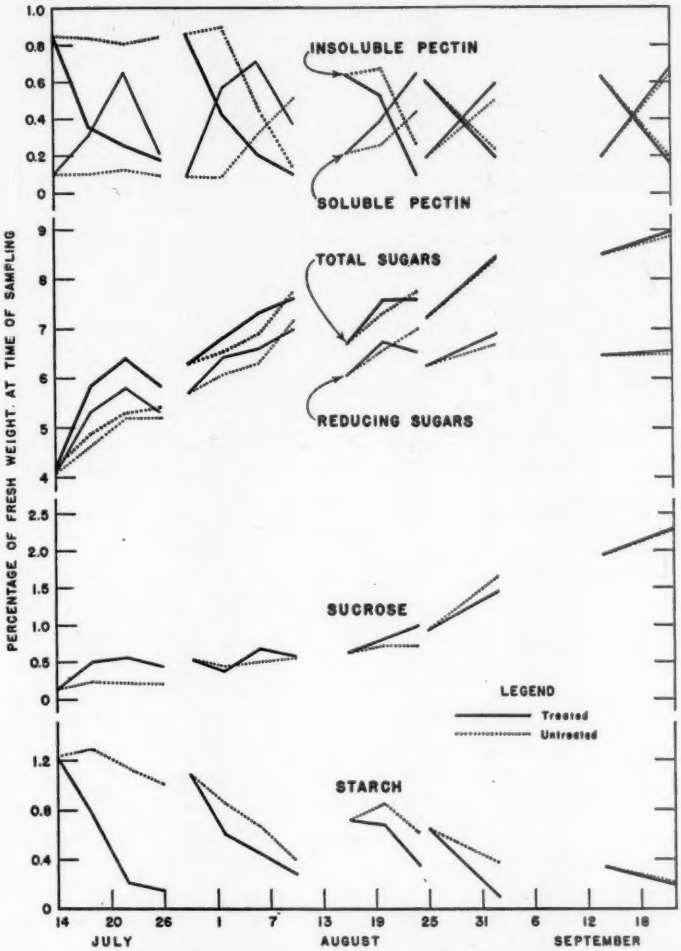


FIG. 1. Effect of ethylene on chemical changes of Bartlett pears picked at different stages of maturity.

during ripening of treated and untreated lots are shown in table III. These values were calculated on the basis of the percentage increase or decrease, at the end of 8 days, over or under the original amount of the material present at time of picking.

CHANGES IN SUGARS.—Total sugars in the green fruit increased throughout the growing season. During ripening, definitely greater increases were

TABLE III

COMPARATIVE CHANGES IN THE CONSTITUENTS OF ETHYLENE-TREATED AND UNTREATED BARTLETT PEARS, EXPRESSED AS THE PERCENTAGE INCREASE OR DECREASE DURING EIGHT DAYS' RIPENING, OF THE AMOUNT ORIGINALLY PRESENT

Date of picking	Increase in total sugars			Increase in reducing sugars			Increase or decrease in sucrose			Increase in soluble pectin			Decrease in insoluble protopectin			Decrease in starch		
	Treated	Untreated	Difference	Treated	Untreated	Difference	Treated	Untreated	Difference	Treated	Untreated	Difference	Treated	Untreated	Difference	Treated	Untreated	Difference
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
7/14	52.15	25.95	26.20	42.86	27.59	15.27	331.00	−15.39	346.4	550.0	20.0	530.0	70.60	5.88	64.72	83.06	8.07	74.99
7/29	16.40	8.60	7.80	15.79	10.53	5.26	21.82	− 9.09	30.91	689.0	255.6	433.4	76.74	46.51	30.23	58.73	39.46	19.27
8/16	13.47	15.57	−2.10	7.95	15.73	−7.78	63.95	14.75	49.20	204.4	100.0	104.4	84.56	59.37	25.19	52.11	15.49	36.62
8/25	17.07	16.78	0.29	10.58	6.89	3.69	56.99	78.49	−21.50	210.6	168.4	42.2	68.85	62.30	6.55	84.39	42.19	42.20
9/14	5.65	4.47	1.18	1.71	0.62	1.09	18.04	16.49	1.55	257.9	247.4	10.5	76.19	69.84	6.35	44.12	38.28	5.89

observed in the ethylene-treated than in the untreated lots in the first and second pickings; but in more mature fruit these differences were much less pronounced. As can be noted in table III, the increase in total sugars during ripening was 26 per cent. greater in the treated fruit of the first picking but only 1.18 per cent. greater in the last picking.

Reducing sugars showed changes similar to those found for total sugars. In the fruit of the first picking the increase in the treated sample was 15.27 per cent. over the untreated. In the final picking, however, an increase of only 1.09 per cent. was found in favor of ethylene treatment.

Very little sucrose occurs in immature green fruit but this sugar builds up constantly during maturation. During ripening more sucrose developed in the treated than in the untreated pears in all but one of the samples collected. In the first picking this increase amounted to over 300 per cent. in favor of ethylene treatment. Much smaller differences were found in fruit picked and ripened later in the season.

STARCH.—The maximum concentration of starch was found in the first sample collected, and the amount contained in the tissues at time of picking decreased thereafter. After ripening of the earliest collected samples, it became evident that ethylene treatment was very effective in increasing the rate of starch hydrolysis. In the treated samples of the first picking practically all of the starch had disappeared after a period of 8 days, while the untreated lot still retained all but a small fraction of the amount originally present. When the last fruit was collected late in the season, there was little starch remaining, and during ripening this disappeared almost as rapidly in the untreated as in the treated fruit.

ALCOHOL INSOLUBLE RESIDUE.—The amount of this material decreased throughout the growing season, and fruit treated early in the season contained less after ripening than did untreated fruit held for a similar period of time. These differences were not noticeable after ripening of more mature fruit picked at later dates.

PECTIC CHANGES.—The amount of soluble pectin in the green fruit at the time of picking was very small, never exceeding 0.2 per cent., even in pears collected two weeks later than the period when commercially mature. After picking and during ripening, the increase in soluble pectin closely parallels the increase in softening of the fruit and is, therefore, a very good index of progress in ripening.

That ethylene has a very pronounced effect on pectic changes is evident from the results obtained. Untreated fruit of the first picking was held for 12 days without any increase in soluble pectin. In the ethylene treated lot, however, there was a very rapid increase which at the end of 8 days amounted to more than 500 per cent. over the amount originally present.

In the untreated sample of the second picking, soluble pectin showed no increase during a 4-day period, but increased rapidly thereafter. In all fruit

collected at later dates, soluble pectin began to increase immediately after picking in the untreated lots, but the rate of this increase was always less rapid than that observed in the treated fruit. Even in the post-mature pears, the amount of soluble pectin developed was higher in the treated fruit, but the difference was much less than that found in earlier picked samples.

The amount of insoluble protopectin in the green fruit decreased slightly throughout the season. During ripening there was a further decrease which occurred more rapidly in the treated fruit. Judging from the data obtained, it is assumed that this decrease in insoluble protopectin is related to the increase in soluble pectin since the percentage decrease in one can approximately be accounted for on analysis as the percentage increase in the other.

The relation of protopectin to pectin has been investigated extensively in apples by CARRÉ (3). APPLEMAN and CONRAD (2) have reported that in peaches the rate of softening parallels the transformation of protopectin into pectin. Similar observations have been made on pears by EMMETT (9).

That ethylene increases the rate of softening in fruit is a common observation. As far as the writer is aware, however, specific data to show the effect of ethylene in increasing the rate of transformation of protopectin to pectin have not been reported previously.

ACID.—The amount of acid found in the Bartlett pears used in these experiments was small, never exceeding 0.3 per cent. During the course of ripening, the acid content showed considerable variation; and no well-defined trends in either treated or untreated lots were apparent. In some lots of fruit titratable acidity appeared to increase during ripening, but in most cases the changes observed were very small.

EXPERIMENTS WITH BARTLETT PEARS RIPENED AFTER STORAGE

The pears used for this series were held at a temperature of 31° F. Samples for analysis were withdrawn after periods of 10, 20, 30, 70, and 120 days, and then ripened at 65° F., as in all previous experiments. The chemical changes occurring in treated and untreated lots during ripening are shown in table IV.

CHANGES IN SUGARS.—Total and reducing sugars increased throughout the storage period. During ripening they showed a tendency to increase, but with the probable exception of the earliest withdrawn samples there were no significant differences observed between treated and untreated lots.

Sucrose increased during the early storage period but declined slightly thereafter. Ethylene treatment did not appear to have any significant effect in increasing or decreasing the concentration over that found in untreated lots after ripening.

STARCH.—Starch was hydrolyzed rapidly during storage. Although microchemical tests showed this material evenly distributed throughout the

TABLE IV

EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARTLETT PEARS RIPENED AFTER STORAGE AT 31° F.

No. days of storage	No. days ripened	Total sugars		Reducing sugars		Sucrose		Soluble pectin		Insoluble protopectin		Starch		Alcohol-insoluble residue		Acid	
		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	0	7.65	7.65	6.79	6.79	0.82	0.82	0.10	0.10	0.78	0.78	0.99	0.99	4.33	4.33	0.19	0.19
	8	9.10	9.20	7.54	7.81	1.48	1.32	0.39	0.57	0.48	0.21	0.30	0.21	3.00	3.06	0.23	0.24
10	0	8.22	8.22	6.72	6.72	1.43	1.43	0.10	0.10	0.61	0.61	0.68	0.68	3.58	3.58	0.19	0.19
	8	9.15	9.15	7.52	7.46	1.54	1.61	0.53	0.59	0.24	0.17	0.27	0.13	3.23	3.15	0.19	0.19
20	0	8.73	8.73	7.56	7.56	1.11	1.11	0.18	0.18	0.73	0.73	0.41	0.41	3.45	3.45	0.22	0.22
	8	8.74	8.96	7.20	7.43	1.46	1.46	0.61	0.69	0.18	0.16	0.23	0.17	3.49	3.53	0.21	0.20
30	0	8.33	8.33	7.34	7.34	0.94	0.94	0.09	0.09	0.87	0.87	0.19	0.19	3.64	3.64	0.18	0.18
	8	9.07	9.11	7.60	7.62	1.40	1.42	0.71	0.79	0.45	0.41	0.16	0.19	3.48	3.57	0.21	0.22
70	0	8.87	8.87	7.92	7.92	0.90	0.90	0.11	0.11	0.75	0.75	0.15	0.15	3.71	3.71	0.20	0.20
	8	9.07	9.03	7.41	7.53	1.57	1.43	0.65	0.64	0.14	0.12	0.15	0.14	3.42	3.42	0.18	0.20
120	0	8.97	8.97	7.92	7.92	0.99	0.99	0.36	0.36	0.37	0.37	0.08	0.08	3.37	3.37	0.21	0.21
	8	9.00	9.03	7.41	7.53	1.51	1.43	0.36	0.37	0.34	0.32	0.07	0.09	2.78	3.00	0.19	0.20

tissues of the fruit at the time of harvest, only traces could be detected after 30 days of cold storage. The hydrolysis of starch, therefore, does not enter into the ripening changes of pears withdrawn after delayed periods of storage. This fact has been observed by MARTIN (22).

During ripening the rate of hydrolysis tended to be more rapid in the treated lots, but starch also disappeared rapidly in the untreated fruit, and the differences observed, therefore, were not as large as those found in newly-picked fruit.

ALCOHOL-INSOLUBLE RESIDUE.—The alcohol-insoluble residue decreased throughout the storage period, and no significant differences were found in the amount of this material remaining in treated and untreated lots after ripening. This would be expected since starch was hydrolyzed almost as rapidly in the untreated as in the treated lots. Other undetermined hydrolyzable materials, such as hemicellulose, would probably respond in a similar manner.

ACID.—The changes in acid during ripening of the storage fruit were very small, and no significant differences were observed between treated and untreated samples.

CHANGES IN PECTIC SUBSTANCES.—Ethylene continued to affect pectic changes for a longer period of time than starch hydrolysis or any other chemical changes observed. In the initial storage sample, protopectin disappeared twice as fast in the treated fruit, and the increase in soluble pectin was almost twice as great. In fruit which had been held for various periods in storage, the rate of protopectin transformation in untreated fruit was more rapid than that observed in pears ripened before storage, and this rate could be increased but little by ethylene treatment. After 30 days of storage, however, slightly more soluble pectin was found in the treated fruit when ripened.

The decrease in effect of ethylene treatment in relation to the increase in maturity of fruit has been observed by ALLEN (1). He found that this gas was more effective in influencing sugar, acid, and starch changes in Gravenstein apples collected early in the season than in those picked at later dates. With pears he also found that the rate of softening and ripening was affected mostly in fruit treated prior to storage. Chemical analysis made after the fruit had been held in storage for 10 and 15 weeks showed no differences in acid or in total and reducing sugars between treated and untreated lots. The suggestion was made, however, that in view of the results on softening and color, it would appear that greater differences in chemical composition might have been found had samples been analyzed a short time after harvesting. The results obtained in the present experiments show clearly that this assumption was correct.

Experiments with Anjou pears ripened after storage

Some studies of the Anjou pear were included because this variety keeps longer and, as shown formerly (**12**), responds to ethylene treatment for a longer period of time after storage than the Bartlett pear. The fruit for this series was held at 31° F., and samples were withdrawn for analysis after 20, 60, and 120 days. In addition to chemical analysis of treated and untreated lots, respiration determinations were made during ripening, using the method of Harding and Maney (**13**). Pressure tests before and after ripen-

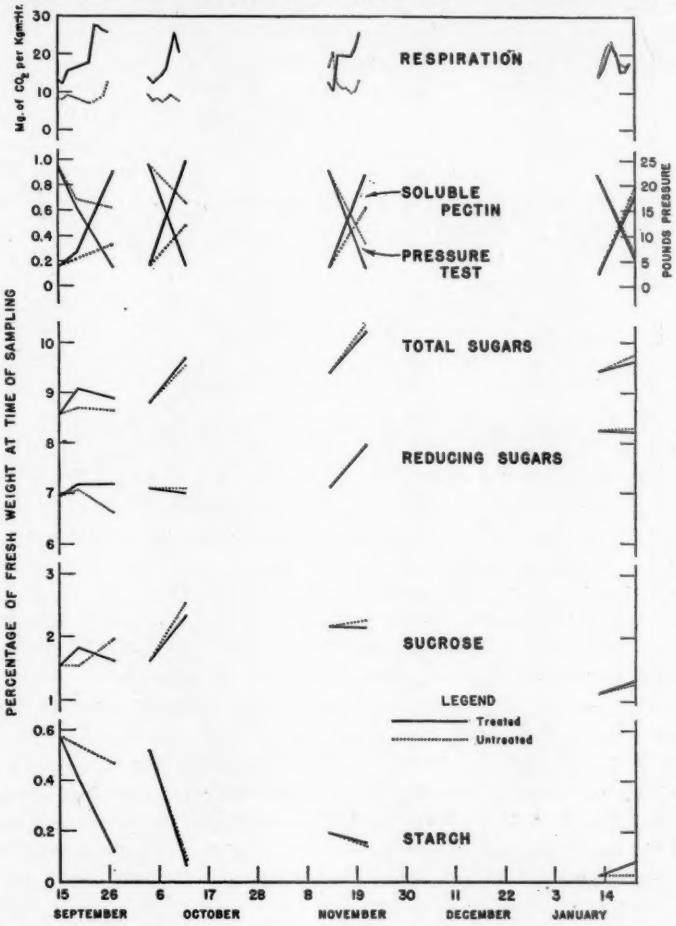


Fig. 2. Effect of ethylene on chemical changes of Anjou pears ripened before and after storage at 31° F.

ing also were made, using the Oregon pressure tester (**23**). The data presented in table V show the comparative chemical composition and the pressure tests of the ethylene-treated and untreated lots before and after ripening. Comparative changes in sugar, starch, pectin, pressure test, and respiration are represented graphically in figure 2.

The results obtained with the Anjou are similar to those found for the Bartlett, and therefore, will not be discussed in detail.

CHEMICAL CHANGES.—The greatest effects from ethylene were obtained early in the storage period. Total and reducing sugars increased more rapidly in the treated lot ripened immediately after harvest; but fruit ripened after being held for various periods at 31° F. showed no differences which could not be attributed to experimental error. In one or two instances sucrose was found to be slightly lower in the treated lots but in most cases the differences were not significant. Since the hydrolysis of sucrose does not occur in the early stages of ripening, as shown by MARTIN (**22**), it is possible that had the ripening process been observed over a longer period, ethylene might have shown more effect on sucrose hydrolysis. This appears likely, since HIBBARD (**16**) has reported that he found less sucrose in several kinds of fruits as a result of ethylene treatment.

Starch disappeared more rapidly in the treated fruit ripened at time of storage, but ethylene had little effect upon increasing the rate of its hydrolysis after the pears had been held at a low temperature for even short periods of time.

In the Anjou pear, as in the Bartlett, the pectic changes were influenced by ethylene treatment for a longer period of time than starch hydrolysis or any other chemical change observed. As can be noted in table V, starch hydrolysis was not affected by ethylene even after only 20 days' storage, although there was still considerable of this material in the fruit tissues at this time. Decidedly higher amounts of soluble pectin, however, developed in the treated fruit ripened at this period, and also in the treated lot ripened 40 days later. The rate of pectic changes, though, was eventually unaffected by ethylene, as shown by similar amounts of soluble pectin found in the untreated and treated fruit ripened after 120 days' storage.

PRESSURE TEST.—The pressure test decreased only slightly during 120 days of storage, indicating that very little softening of the tissues had occurred while the fruit was held at 31° F. Pressure tests of the ethylene-treated lots were much lower than those of the untreated fruit ripened before and after 20 and 60 days of storage, indicating a much more rapid softening of the tissues as a result of treatment. Treated and untreated fruit which was ripened after 120 days' storage showed very little difference in pressure test.

It is apparent from these results that softening of the tissues and the development of soluble pectin are closely correlated. This is indicated by

TABLE V

EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF ANJOU PEARS RIPENED BEFORE AND AFTER STORAGE AT 31° F.

No. days ripened	Total sugars		Reducing sugars		Sucrose		Soluble pectin		Pressure test in pounds		Starch		Alcohol insoluble residue	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*lb.*	*lb.*	*gm.*	*gm.*	*gm.*	*gm.*
When stored														
0	8.57	8.57	6.95	6.95	1.54	1.54	0.14	0.14	23.9	23.9	0.58	0.58	4.51	4.51
4	8.69	9.09	7.07	7.17	1.54	1.82	0.21	0.27	17.0	15.6	0.54	0.41	4.19	4.25
12	8.64	8.90	6.63	7.19	1.96	1.62	0.33	0.92	15.6	3.7	0.47	0.12	4.46	3.97
After 20 days' storage														
0	8.80	8.80	7.08	7.08	1.63	1.63	0.15	0.15	24.0	24.0	0.52	0.52	4.09	4.09
8	9.55	9.70	7.08	7.00	2.34	2.56	0.50	0.99	16.6	4.0	0.07	0.09	3.92	3.90
After 60 days' storage														
0	9.40	9.40	7.11	7.11	2.17	2.17	0.15	0.15	23.0	23.0	0.20	0.20	3.89	3.89
8	10.36	10.23	7.97	7.96	2.27	2.16	0.62	0.89	8.4	3.7	0.16	0.15	4.05	3.89
After 120 days' storage														
0	9.44	9.44	8.25	8.25	1.13	1.13	0.11	0.11	22.2	22.2	0.03	0.03	4.05	4.05
8	9.75	9.65	8.30	8.23	1.28	1.34	0.80	0.73	6.5	5.5	0.03	0.08	3.54	3.79

the fact that higher pectin content was always associated with lower pressure tests in the ethylene-treated fruit as compared to lower pectin content and higher pressure tests in the untreated fruit. This correlation is also indicated by the fact that fruit ripened under treatment after 120 days of storage showed no increase in soluble pectin over that found in untreated fruit; neither did this fruit show any significant decrease in pressure test as a result of similar treatment.

RESPIRATION.—Increases in respiration were obtained long after starch and sugar changes ceased to be affected by ethylene. As can be observed in figure 2, rate of respiration was consistently higher in the ethylene-treated lots ripened before and after 20 and 60 days' storage; but no significant differences were observed between treated and untreated fruit ripened after being held at 31° F. for 120 days. It will be recalled that the development of soluble pectin was also no longer affected by ethylene after a similar storage period, and it is therefore apparent that respiration and pectic changes cease to respond to ethylene treatment at approximately the same time.

Considerable importance has been attributed by KIDD and WEST (**18**) to the increase in respiration occurring prior to ripening in apples. They have applied the term "climacteric" to this respiratory increase, since they consider that it marks a transition to senescence in the life of the fruit. Ripening of the fruit, *i.e.*, softening of the tissue, the development of flavor and aroma, are considered senescent phenomena; since they were observed not to occur in apples until the climacteric had passed.

Various theories have been advanced to account for this increase in respiration observed to take place at a definite period in the life of the fruit. KIDD and WEST (**17**) suggested it was associated with a decrease in acidity; WARDLAW and LEONARD (**25**) to an increase in the internal concentration of oxygen; GUSTAFSON (**11**) to a decrease in hydrogen-ion concentration. The results of the present experiments indicate that the increase in respiration in pears is not associated with a particular concentration of any specific chemical substance, especially sugars. Neither does it appear to be associated with any change in titratable acidity. The fact that this increase in respiratory activity can be initiated so readily with ethylene would indicate that the basic changes brought about by this gas are undoubtedly causal factors of this phenomenon. In addition, the fact that ethylene is so effective in initiating ripening changes other than the climacteric indicate that possibly the basic changes occurring in all cases may be similar in nature. For this reason, further experiments with ethylene, especially in relation to enzyme reactions, appear justified.

Discussion

It is assumed from the results obtained in these investigations with pears that ethylene treatment applied at certain periods in the life of the fruit

definitely affects the principal reactions associated with the ripening processes. This is shown by the increased rate of starch hydrolysis, the higher sugar content, the more rapid transformation of protopectin to pectin and by the increase in respiratory activity in the fruit to which ethylene has been applied. The initiation of these chemical ripenng processes could be brought about by ethylene treatment of pears picked at a very immature stage, long before the fruit had naturally developed to the period when ripening would normally occur. In addition, the reactions occurring in the presence of ethylene were identical to the changes that were observed to take place in mature fruit ripened naturally under normal conditions. In the light of these facts, the question is raised as to what would be the effects of a natural accumulation of ethylene in the tissues of the fruit.

The magnitude of the chemical changes affected by ethylene during ripening is determined by the maturity of the fruit and by the length of time it is held in storage prior to treatment. As previously pointed out, pears picked while still in an early stage of development ripened readily when subjected to ethylene, while similar fruit, not so treated, failed to ripen altogether or was markedly delayed in ripening beyond the period required for treated fruit. In pears collected at more mature stages, the ripening changes naturally progressed at a more rapid rate, and the effect observed from ethylene, therefore, was less pronounced. After the pears had been held in storage for comparatively short periods of time, the chemical changes resulting in ripening occurred very rapidly when the fruit was removed to a higher temperature, and little if any benefit could be observed from the use of ethylene.

The length of time during which the individual chemical changes associated with ripening are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic transformations and respiration. Starch disappears rapidly after picking or storage, and the hydrolysis of this material does not enter into the ripening process of pears withdrawn after long periods of storage. Pectic changes and respiratory activity cease to respond to ethylene at approximately the same period.

Summary

1. Ethylene treatment, when applied at certain periods, was found to increase the rate of starch digestion, the concentration of total and reducing sugars, and the transformation of protopectin to pectin in Bartlett and Anjou pears. No changes in titratable acidity were found.

2. The increase in rate of softening observed in pears treated with ethylene is definitely correlated with an increase in rate of pectic changes.

3. The magnitude of the response obtained with ethylene is determined by the maturity of the fruit and by the length of time held in storage prior to treatment.

4. The length of time during which the individual chemical ripening changes are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic reactions and respiration.

DEPARTMENT OF HORTICULTURE
OREGON AGRICULTURAL EXPERIMENT STATION

LITERATURE CITED

1. ALLEN, F. W. Physical and chemical changes in the ripening of deciduous fruits. Hilgardia **6**: 381–441. 1932.
2. APPLEMAN, C. O., and CONRAD, C. M. Pectic constituents of peaches and their relation to softening of the fruit. Maryland Exp. Sta. Bull. 283. 1926.
3. CARRÉ, MARJORIE H. An investigation of the changes which occur in the pectic constituents of stored fruit. Biochem. Jour. **16**: 704–712. 1922.
4. ————, and HAYNES, D. The estimation of pectin as calcium pectate and the application of this method to the determination of soluble pectin in apples. Biochem. Jour. **16**: 60–69. 1922.
5. CHACE, E. M., and CHURCH, C. G. Effect of ethylene on the composition and color of fruits. Ind. and Eng. Chem. **19**: 1135–1139. 1927.
6. DENNY, F. E. Effect of ethylene upon respiration of lemons. Bot. Gaz. **77**: 322–329. 1924.
7. ————, and MILLER, LAWRENCE P. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. **7**: 97–102. 1935.
8. ELMER, O. H. Growth inhibition in the potato caused by a gas emanating from apples. Jour. Agr. Res. **52**: 609–626. 1936.
9. EMMETT, A. M. An investigation of the changes which take place in the chemical composition of pears stored at different temperatures, with special reference to pectic changes. Ann. Bot. **43**: 269–308. 1929.
10. GANE, R. The formation of ethylene by plant tissues, and its significance in the ripening of fruits. Jour. Pomol. **13**: 351–358. 1935.
11. GUSTAFSON, F. G. Growth studies on fruits. Respiration of tomato fruits. Plant Physiol. **4**: 349–356. 1929.
12. HANSEN, ELMER, and HARTMAN, HENRY. Effect of ethylene and certain metabolic gases upon respiration and ripening of pears before and after cold storage. Plant Physiol. **12**: 441–454. 1937.
13. HARDING, P. L., MANEY, T. J., and PLAGGE, H. H. Apparatus for the determination of carbon dioxide in the respiration of apples. Science n.s. **70**: 125–126. 1929.
14. HARVEY, R. B. Artificial ripening of fruits and vegetables. Univ. Minnesota Agr. Exp. Sta. Bull. 247. 1928.

15. ————————. Ethylene as a ripener of fruits and vegetables. Science n.s. **67**: 421–422. 1928.
16. HIBBARD, R. P. The physiological effect of ethylene gas upon celery, tomatoes, and certain fruits. Michigan Sta. Tech. Bull. 104. 1930.
17. KIDD, F. W. Respiration of fruits. Nature **135**: 3409. 1935.
18. ————————, and WEST, C. The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Great Britain Dept. Sci. and Ind. Res. Food Invst. Bd. Rpt. pp. 51–57. 1933.
19. LANE, J. H., and EYNON, L. Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. Soc. Chem. Ind. Trans. **42**: 32T–37T. 1923.
20. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill Book Co. 1937.
21. MARTIN, WM. E. Distribution of certain sugars in Bosc pears. Plant Physiol. **11**: 139–147. 1936.
22. ————————. Chemical study of the ripening process of Bosc pears. Bot. Gaz. **99**: 42–68. 1937.
23. MURNEEK, A. E. A new test for the maturity of the pear. Oregon Agr. Exp. Sta. Bull. 186. 1921.
24. SHAFFER, P. A., and HARTMANN, A. F. The iodimetric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk, and other solutions. Jour. Biol. Chem. **45**: 365–390. 1921.
25. WARDLAW, C. W., and LEONARD, E. R. Studies in tropical fruits. I. Preliminary observations on some aspects of development, ripening and senescence, with special reference to respiration. Ann. Bot. **50**: 621–653. 1936.
26. WOLFE, HERBERT. Effect of ethylene on ripening of bananas. Bot. Gaz. **92**: 337–366. 1931.
27. WORK, PAUL. Ethylene ripening of tomatoes in relation to stage of maturity. Proc. Amer. Soc. Hort. Sci. **25**: 61–65. 1928.

GROWTH BEHAVIOR OF ONE-MILLIMETER EXCISED ROOT TIPS

GLADYS C. GALLIGAR

(WITH ONE FIGURE)

Introduction

In 1893 RECHINGER (**5**) attempted to find the degree of divisibility of plant parts beyond which no growth could take place. He concluded that the presence of vascular strands is necessary to the growth of isolated plant tissue. In 1922 KOTTE (**7**) refuted this conclusion, which had been corroborated by LAMPRECHT in 1918, by securing as many as six centimeters of growth from one millimeter root tips (including the cap). Since then WHITE (**11**) has found that the terminal one tenth millimeter detached from a root retains the capacity to differentiate into regions of root cap, epidermis, cortex, and vascular strand. The present investigation was undertaken more for comparison with previous work of the author, in which the original length of the root tips was 10 mm., rather than as a confirmation of KOTTE'S work.

Materials and methods

The procedure used to secure sterile root tips has been outlined in previous papers (**4, 5**).

Root tips, one millimeter in length, excised from seedlings of dent corn, sunflower, sweet corn, cotton, Gradus pea, and Burpee's Extra Early pea were included in the first series of experiments. The growth of the root tips of cotton and the two varieties of pea was so poor (table I) that these plants were discontinued in subsequent series.

The nutrient solution was a modification of Pfeffer's formula to which dextrose and peptone were added.[1] Immediately after it was made, the

[1] $Ca(NO_3)_2$	2.0 gm.	KCl	0.25 gm.	Dextrose	2.0 per cent.
KH_2PO_4	0.5 gm.	$MgSO_4$	0.5 gm.	Peptone	0.04 per cent.
KNO_3	0.5 gm.	$FeCl_3$	0.005 gm.	Distilled H_2O	6000 cc.

solution was measured into the flasks, which were closed with cotton plugs and autoclaved at 15 pounds pressure for 20 minutes.

Discussion of results

The data of table I show marked irregularities of elongation, a condition found also in 10-mm. root tips (**4**). There are no smooth curves of growth, but rather a series of irregular peaks representing spurts of growth at various times. Within the first ten days sunflower and corn grew almost equally

TABLE I

SAMPLE SERIES OF FIVE INDIVIDUALS OF EACH SPECIES SHOWING DAILY INCREMENTS OF GROWTH IN MILLIMETERS FOR EACH INDIVIDUAL DURING THE MEASURABLE STAGE

Burpee's Extra Early pea

1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.5	0	0.5	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0.5	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Sweet corn

1	0	0.5	0	0.5	0.5	0.5	2	1	2	2	2	0	2	1	0	1	1	1	2	0	2	1	1	2	1	1	1	0	0	...	...	...	...	...	...	...
2	0	0.5	0.5	0	1	0	1	1	2	2	1	1	1	3	1	1	1	1	2	2	0	2	1	3	2	0	2	6	1	...	...	...	...	...	...	...
3	0	0.5	1	0	0.5	1	1	2	2	2.5	1.5	0.5	1	3	1	1	2	2	2	2	2	2	1	3	2	2	2	...	...	...	...	...	...	...	...
4	0	0.5	1	0.5	1	1	2	3	1	2	2	2	0	1	1	2	1	1	1	3	1	1	3	3	2	3	3	2	2	...	...	...	...	...	...	...
5	0.5	0.5	0.5	1.5	1.5	1.5	1.5	2.5	2	2	1	3	1	2	1	1	1	2	1	0	2	1	2	2	2	1	2	2	2	...	...	...	...	...	...	...

Corn

1	1	0	0	0	0.5	0.5	3	7	3	5	3	3	2	2	4	1	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2	0.5	0	0.5	0	0	0.5	1.5	2	2	6	5	4	7	8	4	13	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	1	0	0	0.5	0.5	1	2	4	3	2	1	1	3	3	0	2	0	1	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4	0.5	0	0.5	0	1	1	5	8	5	7	7	11	4	3	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
5	1	0	0.5	0.5	4	3	5	7	8	4	3	3	6	6	4	...	...	...	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Sunflower

1	0.5	0	0.5	1	0	1	0	1	1	1	4	3	0	1	4	1	1	1	3	1	1	0	0	2	2	0	1	0	0	0	0	0	4	7	2	2
2	0	0.5	0.5	1	0	1.5	0.5	1	2	1	3	1	2	2	3	0	0	0	0	1	2	0	0	0	0	1	0	1	0	0	0	0	2	1	1	2
3	1.5	0.5	2	2	1	2	3	4	3	1	1	1	2	2	0	1	1	1	2	1	2	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0
4	0	0	0	0.5	0	0.5	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0.5	1.5	0	0.5	1.5	1	1	3	1	1	1	0	2	0	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0

Cotton

1	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0.5	0.5	0.5	0	0.5	1.5	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0.5	0.5	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Gradus pea

1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

well while sweet corn was not far behind. But insignificant elongation occurred in cotton, Gradus pea, and Burpee's Extra Early pea.

As a rule the daily rate of growth for the first few days was very slow as compared with that of the 10-mm. root tips of the same species. A faster growth rate, approaching that of the 10-mm. root tips, was attained from the seventh to the tenth day. The slow initial growth may be accounted for by the delayed recovery from the injury of excision. In a 10-mm. root tip the ratio of wounded tissue to the whole surface of the root fragment is insignificant when compared to that of a 1-mm. root tip. In the latter case the wounded area is quite large in relation to the whole surface of the root. The wound is also in a region of high metabolic activity, where the shock resulting from such a wound is much greater than the shock from a wound several millimeters removed from the actively growing meristem. Consequently a slower growth rate may be expected from the shorter fragments during the first few days. Another factor causing more rapid initial growth in the longer fragments may be the greater store of natural reserves of food in their mature tissues.

The data on sunflower, dent corn, and sweet corn are summarized in table II. These figures offer opportunity for interesting comparisons with the results of the 10-mm. root tips (**4**). All three varieties of root tips cut at 1 mm. are alike in having average total gains in length within the first ten days that fall far short of the corresponding gains for roots having an initial length of 10 mm. One-millimeter root tips of dent corn and sweet corn also fall short in the figures on final total length, range in total length, average number of secondary roots, range in number of secondary roots, and average dry weight for the 10-mm. root tips. Strangely enough, final data on 1-mm. sunflower root tips exceeded those of 10-mm. root tips, showing that sunflower performed much better unencumbered with several millimeters of mature tissue in initial stages. This is diametrically opposed to RECHINGER'S view (**5**) that differentiated vascular tissue is essential to the growth of isolated plant parts.

A comparison of 1-mm. root tips of the different species with each other discloses some interesting facts (table II). The data on the average values for sweet corn were lowest throughout except in the case of dry weight, which was slightly greater than that of corn, indicating that sweet corn was more able to attain its growth in length at less expense of its own reserves. The reverse was true when the original lengths of sweet corn were 10 mm. (**4**). Sunflower was superior to corn and sweet corn in every respect except in average increment in length the first ten days and in average number of branches. The appearance of secondary roots occurred from the thirteenth to the sixteenth day in dent corn, sweet corn and sunflower,

TABLE II

A SUMMARY OF THE DATA ON 105 ROOT TIPS CUT AT ONE MILLIMETER, INCLUDING THE ROOT CAP, AND GROWN IN THE DARK IN THE MODIFIED PFEFFER'S SOLUTION PLUS 2 PER CENT. DEXTROSE AND 0.04 PER CENT. PEPTONE

SPECIES	AV. DAILY INCREASE IN MM. OF LENGTH OF 30 ROOT TIPS FOR FIRST 10 DAYS										AV. TOTAL LENGTH AT END OF 10 DAYS	AV. FINAL TOTAL LENGTH	RANGE IN FINAL LENGTHS AMONG INDIVIDUALS	FINAL AV. NO. LATERAL ROOTS	RANGE IN FINAL NO. LATERAL ROOTS AMONG INDIVIDUALS	AV. DRY WT. PER 10 ROOTS
	1	2	3	4	5	6	7	8	9	10						
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*			*gm.*
Sunflower	0.55	0.63	0.54	0.59	0.67	1.68	1.25	1.79	1.20	1.42	10.32	121	43–283	35	0–89	0.283
Sweet corn	0.33	0.30	0.43	0.85	0.15	0.94	1.32	2.00	1.60	1.61	9.53	85	33–155	13	0–69	0.078
Corn	0.40	0.25	0.31	0.45	0.88	0.85	1.92	3.05	2.20	2.50	12.81	89	24–189	38	5–61	0.074

somewhat later than in the longer initial fragments, while Gradus pea, Burpee's Extra Early pea and cotton never produced laterals. Several one millimeter fragments of corn, sunflower, and sweet corn appeared to cease growth for as long as twelve to fifteen days, after which growth was resumed

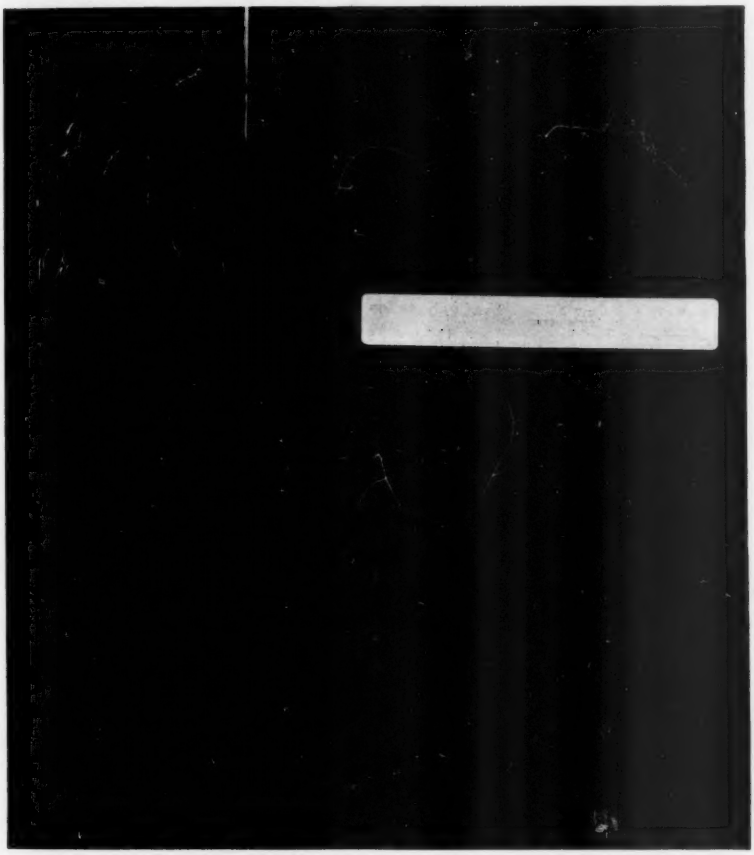


FIG. 1. Growth habits of root tips cut off at 1 mm. and allowed to grow undisturbed in Pfeffer's solution plus glucose and peptone.

Upper left, sunflower root after four months. Note the usual papillae; root hairs are visible near the growing tips.

Upper right, four sweet corn roots after four months. Note the corkscrew twist in the two small ones.

Lower center, a single corn root after four months. Note the extremely fine and ramifying threadlike lateral roots.

or secondaries were produced which grew to great length. This phenomenon was most marked in sunflower, but was also noticeable in corn and sweet corn.

As may be seen in figure 1 each species exhibited certain peculiarities of its own during the process of growth. From 40–50 per cent. of the root tips of dent corn manifested a tendency toward the development of a very short main root axis and a very decided development of laterals. Figure 1 shows a corn root 157 mm. in length with 54 secondary roots, nine of which range from 104–217 mm. in length. This single individual is sufficient evidence that excised root tip meristem of 1-mm. length, including the root cap and devoid of demonstrable differentiated vascular tissue, is capable of enormous growth and development.

On the other hand sweet corn manifested a strong tendency toward development of the main axis of the root and suppression of laterals. About 40 per cent. of the roots developed no laterals at all; about 10 per cent. produced laterals profusely; the rest developed only a few short laterals. Often the roots of sweet corn grew in tight corkscrew spirals (fig. 1), never attaining any great length in such cases. This behavior may have been caused by an injury in excision to one segment of the meristem initials. Occasionally from such spiral roots very fine thread-like laterals grew to lengths of 15 mm. or more (fig. 1).

Except for an initial slower rate of elongation, the 1-mm. tips of sunflower behaved like the 10-mm. tips (**4**). In a few root tips a curious hypertrophy of cells occurred, in which there was no normal elongation, but merely a proliferation of a mass of undifferentiated parenchyma-like cells, similar to the growths secured by WHITE (**11**) on the integumental cells of the seed primordia of *Antirrhinum majus*. The cell masses attained the size of a large pea. The individual cells seemed not to be firmly held together as in ordinary parenchyma.

Summary

1. There was no regularity of growth in excised root tips having an original length of 1 mm.

2. The daily rate of elongation within the first ten days was much slower than that of root tips cut at 10 mm.

3. Within the first ten days sunflower and corn surpassed sweet corn in rapidity of elongation, while cotton, Gradus pea, and Burpee's Extra Early pea grew scarcely at all.

4. Corn showed a tendency to develop a short root with an abundance of laterals, while sweet corn manifested the reverse condition.

5. The presence of vascular tissue was not necessary to the growth of excised root tips.

6. One-millimeter sunflower root tips achieved greater growth than 10-mm root tips.

7. Corn root tips were less able to accumulate dry weight when cut at 1 mm. than when cut at 10 mm.

JAMES MILLIKIN UNIVERSITY
DECATUR, ILLINOIS

LITERATURE CITED

1. BÖRGER, H. Über die Kultur von isolierten Zellen und Gewebsfragmenten. Arch. exp. Zellf. **2**: 123–190. 1926.
2. CHAMBERS, W. H. Cultures of plant cells. Proc. Soc. Exp. Biol. and Med. **21**: 71–72. 1923.
3. CZECH, HELENE. Kultur von pflanzlichen Gewebszellen. Arch. exp. Zellf. **3**: 176–200. 1926.
4. GALLIGAR, GLADYS C. Correlation between growth of excised root tips and types of food stored in the seed. Plant Physiol. **13**: 599–609. 1938.
5. ————. Influence of certain organic substances upon the growth behavior of excised root tips. Trans. Ill. Acad. Sci. **29**: 59–68. 1936.
6. HARRISON, R. G. On the status and significance of tissue culture. Arch. exp. Zellf. **6**: 4–27. 1928.
7. KORSCHELT, E. Regeneration und Transplantation. Gebrüder Borntraeger. Berlin, 1927.
8. KOTTE, W. Kulturversuche mit isolierten Wurzeln. Beitr. allg. Bot. **2**: 413–434. 1922.
9. ————. Wurzelmeristem in Gewebekultur. Ber. d. Bot. Ges. **40**: 269–272. 1922.
10. ROBBINS, W. J. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot. Gaz. **74**: 59–79. 1922.
11. WHITE, P. R. Plant tissue cultures. Arch. exp. Zellf. **10**: 501–515. 1930–31.
12. ————. Plant tissue cultures. Arch. exp. Zellf. **12**: 602–620. 1931–32.
13. ————. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid media. Plant Physiol. **7**: 613–628. 1932.

TRANSPIRATION AND THE ABSORPTION OF MINERAL SALTS[1]

KENNETH E. WRIGHT

Introduction

The effect of varying rates of transpiration upon the absorption of mineral salts is an old question that has been studied by numerous investigators. No attempt will be made here to review these bulky data. Earlier opinions, as summarized by CURTIS (**2**), were usually in favor of increased absorption of mineral salts. More recent studies by various investigators are characterized by a decided lack of unanimity of opinion. The work of HASSELBRING (**7**), KIESSELBACH (**9**), MUENSCHER (**12**), MENDIOLA (**10**) and others indicates that different rates of transpiration are without effect upon the absorption of mineral salts. Investigations conducted by HASS and REED (**6**), HITCHCOCK and ZIMMERMAN (**8**), and FREELAND (**3, 4**) support the contention that transpiration is a factor which affects the absorption and translocation of mineral salts.

The confusion that exists in this field of investigation is probably caused largely by the failure to eliminate all other factors in the metabolism of the plant, which may affect the absorption of mineral salts. It is the purpose of this paper to present a technique that will predominantly keep these other contributing factors constant, and, with such controlled conditions, to demonstrate that different rates of transpiration are accompanied by corresponding rates in the absorption of certain anions and cations.

Methods

Seeds of *Phaseolus vulgaris,* var. *humilis,* bush bean, were germinated in distilled water and grown to the flowering stage in HARTWELL and PEMBER'S (**5**) culture solution. Six plants were then transferred to a round battery jar which had a disc cut from ply board to fit the top, with holes bored through it to receive the plants. The wooden disc was paraffined to prevent water loss. A wide overlapping rubber band held the paraffined disc on the top of the jar, and cotton plugs maintained the plants in position. The interior of the jar was kept dark by a coating of black asphalt paint over the outside surface of the jar. Each of the several jars was filled with 2000 cc. of culture solution. The entire system was then weighed, and allowed to stand in the greenhouse for ten days. At the end of this period all of the jars were again weighed. Two were selected that had lost the same weight of water measured in grams. These jars were subsequently

[1] Published by permission of the Director of Research, as contribution no. 525 of the Rhode Island Agricultural Experiment Station.

filled with 2000 cc. of culture solution and placed in a high and low humidity cabinet.

This cabinet was made of window glass, except the wooden floor and the framework, and was divided into two compartments by a glass partition. In one chamber humidity was kept high by means of a pan of water, and by water vapor given off by the transpiring plants. In the other chamber a low humidity was obtained by connecting this chamber in a circuit with a Frigidaire freezing unit, and forcing air through the circuit with an air pump driven by an electric motor. The air pump was located in the cold air portion of the circuit between the freezing unit and the cabinet. By the time the cold air had gone through the pump and the remaining circuit its temperature was the same as that of the high humidity chamber. This circuit was a closed system, thus eliminating the possibility of a fresh supply of carbon dioxide accelerating the rate of photosynthesis with a consequent increase in the supply of sugars to the root system. The volume of the humid chamber was 8.06 cu. ft., and that of the dry chamber, because of the additional space in the freezing unit, was 8.78 cu. ft. This difference was considered sufficiently small to have little significance in affecting the sugar supply, and consequently the absorption capacity of the roots.

Circulating air, as opposed to still air, should have no effect on the aeration of the culture solutions, as the containers were airtight with the exception of compact cotton plugs which held the plants in position in the paraffined top of the container.

The cabinet was placed in the greenhouse with a cloth screen in position between the cabinet and the direct rays of the sun to prevent the temperature inside the cabinet from rising above that of the greenhouse.

The plants were in the cabinet for ninety-six hours, and then the remaining culture solution in each jar was measured and retained for analysis. The jars were refilled with 2000 cc. of culture solution and were replaced in the cabinet for another run, but in this second run the plants that had previously been in the high humidity chamber were placed in the low humidity chamber. Those that had been in the low humidity side of the cabinet were placed in the high humidity side. At the conclusion of a ninety-six hour run the residual culture solutions were again measured and saved for analysis.

The original culture solution and the residual culture solutions were analyzed for calcium and for nitrates according to the standard methods as described by the Association of Official Agricultural Chemists (1). Phosphorus was determined by the colorimetric method of TRUOG and MEYER (13), and potassium by the colorimetric method as described by MORRIS and GERDEL (11).

Results and discussion

The results as recorded in table I are typical of two other runs made with different sets of plants at different times of the year. An examination

TABLE I

AMOUNT OF MINERAL ABSORPTION WITH HIGH TRANSPIRATION AND LOW TRANSPIRATION

TREATMENT	WATER ABSORBED	PHOSPHORUS ABSORBED	CALCIUM ABSORBED	NITRATES ABSORBED	POTASSIUM ABSORBED
	cc.	*mg.*	*mg.*	*mg.*	*mg.*
Jar 1—high transpiration ...	330	13.6	25.0	41.4	35.6
Jar 2—low transpiration	150	8.6	15.0	41.0	27.8
Jar 2—high transpiration ...	335	11.2	27.0	46.8	56.4
Jar 1—low transpiration	165	9.6	13.0	41.8	52.8

of the table demonstrates that in all cases a higher rate of transpiration is accompanied by an increased absorption of the various ions. It is admitted that in any one specific instance the difference in absorption of a certain ion under conditions of high transpiration and of low transpiration is insignificant. However, it is emphasized that a summation of all the analytical evidence illustrates a definite trend favoring increased absorption of minerals under conditions of increased transpiration.

Selecting for experimentation two jars of plants that in the greenhouse lost the same amount of water over a 10 day period, infers a root-shoot balance with approximately the same rate of absorption in both jars of plants. Reversing the positions of the plants in the chambers of the cabinet serves to counteract the effect of varying metabolic processes associated with different sets of plants. With such a procedure it was found that the plants in the high transpiration chamber continued to absorb a greater amount of minerals, which indicates that transpiration is evidently a correlated factor in determining the amount of minerals absorbed.

It is believed that the data here presented substantially support the contention that transpiration is a contributing factor in determining the rate of absorption of mineral ions. The writer does not wish to promulgate the idea that transpiration has a function in the absorption of soil minerals, since, given sufficient minerals in the soil, the laws of diffusion would insure adequate mineral absorption without transpiration. However, it is logical to assume from the data here recorded that increased mineral absorption may be the result of increased transpiration.

Summary

The effect of transpiration upon the absorption of mineral ions was determined by analyzing culture solutions in which bean plants[2] had been

[2] The plants were grown in the culture solutions by WALTER COLVIN and VLADIMIR SHUTAK.

growing under conditions of high and low transpiration. The contributing effects of the metabolism of the plants upon mineral absorption were offset by: (1) selecting plants with the same transpiring power when in the same environment; (2) analyzing the residual culture solutions of the same plants under conditions of high and low transpiration; and (3) devising an experimental procedure attempting to make transpiration the only variable. Analytical results indicate that an increase in the rate of transpiration is associated with a corresponding increase in the absorption of certain ions.

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis, Washington, D. C., 3rd ed. 1930.
2. Curtis, O. F. Translocation of solutes in plants. New York. 1935.
3. Freeland, R. O. Effect of transpiration upon the absorption and distribution of mineral salts in plants. Amer. Jour. Bot. **23**: 355–362. 1936.
4. ————————. Effect of transpiration upon the absorption of mineral salts. Amer. Jour. Bot. **24**: 373–374. 1937.
5. Hartwell, Burt L., and Pember, F. R. The presence of aluminum as a reason for the difference in the effect of so called acid soil on barley and rye. Soil Sci. **6**: 259–279. 1918.
6. Hass, A. R. C., and Reed, H. S. Relation of desiccating winds to fluctuations in ash content of citrus leaves and phenomena of mottle-leaf. Bot. Gaz. **83**: 161–172. 1927.
7. Hasselbring, H. The effect of shading on transpiration and assimilation of the tobacco plant in Cuba. Bot. Gaz. **57**: 257–286. 1914.
8. Hitchcock, A. E., and Zimmerman, P. W. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. **7**: 447–476. 1935.
9. Kiesselbach, T. A. Transpiration as a factor in crop production. Nebraska Agr. Exp. Sta. Res. Bull. **6**: 170–184. 1916.
10. Mendiola, N. B. Effect of different rates of transpiration on the dry weight and ash content of the tobacco plant. Philippine Jour. Sci. **20**: 639–655. 1922.
11. Morris, V. H., and Gerdel, R. W. Rapid colorimetric determination of potassium in plant tissues. Plant Physiol. **8**: 315–319. 1933.
12. Muenscher, W. C. Effect of transpiration on the absorption of salts by plants. Amer. Jour. Bot. **9**: 311–330. 1922.
13. Truog, Emil, and Meyer, A. H. Improvements in the Denigés colorimetric method for phosphorus and arsenic. Ind. & Eng. Chem. Anal. Ed. **1**: 136–139. 1929.

BRIEF PAPERS

TIME AND TEMPERATURE OF PROTOPLASMIC COAGULATION[1]

HENRY T. NORTHEN AND REBECCA TYSON NORTHEN

In most plants protoplasmic activity cannot be maintained at temperatures much above 40° C. The cessation of many vital phenomena at temperatures above or slightly below 40° C. is probably caused by the coagulation of the protoplasm. The data presented will show that temperatures of 43.5° C., and above, cause a decided increase in protoplasmic consistency, which has been interpreted as coagulation, in cells of corn, oat, rye, and wheat coleoptiles and that in *Spirogyra* and *Zygnema* a lower temperature effects a decided increase in protoplasmic firmness.

Filaments of *Spirogyra* and *Zygnema* and one week old corn, oat, rye, and wheat seedlings were immersed for various periods of time in tap water maintained at various temperatures. After the desired immersions the plants were transferred to water at room temperature where they were allowed to remain for twenty minutes. The root systems of the monocots were then removed and the shoots and filaments were centrifuged with an acceleration of 680 X gravity for one minute. After centrifugation the filaments and the coleoptiles were mounted in aceto-carmine and the respective number of cells in which the chloroplasts had been moved by the acceleration was determined. In the coleoptiles the subepidermal cells near the tip and adjacent to the veins were selected for study.

The data are summarized in table I.

In the table when two time figures (*e.g.*, 6–8) are given the actual time necessary to cause coagulation, as evidenced by no displacement in 90 per cent. of the cells, lies between the two values. When only one figure is recorded it indicates the time necessary to cause coagulation in 90 per cent. of the cells.

It is probable that *Spirogyra* no. 1 and *Spirogyra* no. 2 are the same species. Vegetatively they looked alike. *Spirogyra* no. 1 was collected in April when part of the pond was still covered with ice and when the temperature of the water was 7° C., whereas no. 2 was collected in June when the temperature was 18° C. It will be noted that no. 1 coagulated at a much lower temperature than no. 2 and that at 43° C. less than one minute was required to cause coagulation in 90 per cent. of the cells of no. 1, whereas between six and twelve minutes were required to cause coagulation in 90 per

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 168.

TABLE I

TIME AND TEMPERATURE REQUIRED FOR PROTOPLASMIC COAGULATION

PLANT	TEMPERATURE	MINUTES IMMERSION NECESSARY TO PREVENT CENTRIFUGAL ACCELERATION FROM DISPLACING THE CHLOROPLASTS IN 90% OF THE CELLS
	0° C.	*minutes*
Spirogyra no. 1	43.0	< 1
	40.0	< 1
	38.5	< 1
	36.0	2
	33.5	30
Spirogyra no. 2	43.0	6–12
	38.0	*(15%)
Zygnema sp.	43.0	< 1
	40.0	2
	38.5	4
	36.0	12
	33.5	*(26%)
Corn	47.5	6–8
	43.5	30
	40.0	*(0%)
Oats	47.5	1–2
	43.5	15
	40.0	*(0%)
Rye	47.5	2–4
	43.5	15–20
	40.0	*(0%)
Wheat	47.5	2–4
	43.5	12–15
	40.0	*(0%)

* Coagulation had not occurred in most cells after an immersion of forty minutes. The percentages of cells in which coagulation had occurred, as evidenced by a failure of centrifugal acceleration to displace the chloroplasts, are shown in parentheses.

cent. of no. 2. If they were the same species, which seems likely, then the temperature at which they were growing greatly affected the time and temperature of coagulation. The *Zygnema* was collected with *Spirogyra* no. 1 and it also coagulated at a comparatively low temperature.

Furthermore, the data indicate that at temperatures of 43.5° C., and above, the protoplasm in cells of corn, oat, rye, and wheat coleoptiles coagulates. The time necessary to cause coagulation at 43.5° C. and 47.5° C. suggests that the protoplasm in coleoptile cells of wheat, rye, and oats is more sensitive to high temperature than is the protoplasm in coleoptile cells of corn.

UNIVERSITY OF WYOMING
LARAMIE, WYOMING

A METHOD FOR THE TREATMENT OF CUTTINGS AND ROOTS OF THE PECAN WITH ROOT-INDUCING CHEMICALS

C. L. Smith and L. D. Romberg

In preliminary experiments in the rooting of pecan root cuttings it was found that both water solutions and lanolin mixtures of indolebutyric acid were effective in inducing root formation. It was also found that lanolin mixtures of the chemical applied to the bark of root cuttings larger than ½ inch in diameter were less effective in inducing root formation than applications made in slits through the bark or in holes bored laterally into the roots. This was apparently due to the inability of the indolebutyric acid to diffuse through the thick bark. In the large roots it was indicated that a localized treatment was more effective than where the entire base of the cutting was treated as by immersing in a water solution. Preliminary work in 1937 also indicated that the indolebutyric acid was effective in inducing root formation in transplanted pecan trees when it was applied in lanolin mixtures in slits or holes in the roots at the time of transplanting.

Since the large root cuttings responded better to localized treatments, and since it was impractical to use water solutions in the treatment of the roots of transplanted trees, the lanolin mixture of indolebutyric acid was applied in holes or slits in the roots by means of a hypodermic syringe. This method was effective but was not satisfactory because of the difficulty of filling the small holes with the lanolin mixture, and because of the waste of material. A method was devised, therefore, which eliminates both these difficulties and also saves time in the operation.

Ordinary round wooden toothpicks are used as the carriers for the chemical instead of lanolin. The indolebutyric acid is dissolved in 95 per cent. ethyl alcohol in the concentration desired. The two ends of each toothpick are cut off leaving the pick 4 cm. long. Then the picks are immersed in the alcoholic solution for 24 hours, after which they are removed and the alcohol allowed to evaporate. Each pick absorbs approximately 0.084 ml. of the solution, and any desired concentration may be obtained by properly adjusting the concentration of the chemical in the alcohol. The picks also absorb approximately the same volume of water as of alcohol, and at approximately the same rate. The chemical, therefore, readily diffuses from the toothpicks when they are placed in the roots. Holes 7/64 inch in diameter are bored transversely about two-thirds through the cuttings or roots at the desired points by means of an electric drill, and treated toothpicks of sufficient length to fill the holes are inserted.

The treated picks have been as effective as the lanolin mixture in inducing root formation in cuttings, and have been effective in concentrations vary-

ing from 0.25 to 4 mg. per pick; but it appears that the concentration of 4 mg. per pick is too high, since some injury has occurred from its use. Further work is being done on concentrations, on effectiveness of the treatments on the rooting of cuttings, and on the reestablishment of the root systems of transplanted pecan trees.

BUREAU OF PLANT INDUSTRY
U. S. DEPARTMENT OF AGRICULTURE
AUSTIN, TEXAS

PLANT PHYSIOLOGY IN THE WOMEN'S COLLEGES[1]

DOROTHY DAY

A composite picture of the women's colleges shows that each offers one or two courses in plant physiology, generally only one in the physiology of the higher plants. Such courses are usually for juniors and seniors although sophomores or graduate students may be included. They may meet for one-half year or one year, but this in no way indicates that more or longer courses are any better than one short, well-taught course.

Classes in plant physiology are relatively small, often about four students, partly because of the keen competition of the social sciences and liberal arts. Laboratory space is not a factor governing the type of work, although planning seems to be required in some cases. Lack of apparatus becomes a problem only when it is complex or expensive. Individual work is preferred, especially for simpler problems, but often two students to an experiment is necessary to conserve apparatus. May it not be that two students paired wisely, perhaps a chemist and botanist as Dr. LYON suggested last year, might stimulate each other to good advantage? Although stock solutions may be prepared by the instructor or an assistant, it is considered advisable for the student to make some of them, as emphasis on laboratory technique and cleanliness is training that reflects on the college after graduation.

In the recording of experiments variation is great, but there is a hint of informality rather than adherence to rigid specifications. There is clear emphasis, however, on accurate recording of observations with proper discussion or explanation. Student results do not always agree with theory. This may mean that students work until results are obtained which check with accepted theory, or more often they are required to account, if possible, for the deviation. Sometimes this helps them to understand how their inadequate technique may cause errors. Too often they have high aspirations which are never realized because of their inexperience. Errors from lack of practice and from poor technique may blast their high hopes but may also bring a greater appreciation of fine critical research.

The living plant is used more than physical and chemical illustrations. Prerequisites emphasize the botanical background more than the chemical, and perhaps do this to a greater extent than in the large universities. They include elementary botany, plus a second course in botany in several cases. Chemistry as a prerequisite varies through all degrees from none at all to the advice that "some knowledge of organic chemistry is desirable"; or a

[1] Summarized from the report at the Richmond Meeting of the American Society of Plant Physiologists, December 30, 1938.

Contributions from the Department of Botany, Smith College, New Series, no. 3.

year of chemistry or physics may be recommended. The advanced course requires more preparation, usually the elementary physiology plus more botany or chemistry or both. By this scheme of greater emphasis on the botanical background for physiology, the graduates of our women's colleges come to the university well prepared for advanced work and later take important positions in plant physiology.

Support for physiology is mostly from majors in the botany department, not from students in horticulture, for there is no graduate or professional training school to lend strength. Physiology is not required for the major in botany in more than one or two colleges. It is difficult for girls in the various other sciences to meet all the prerequisites. Physiology is not regularly elected by students of horticulture, as they generally lack even elementary chemistry and are usually more interested in the art aspect of landscaping. Several instructors in horticulture and in landscape architecture in these colleges have expressed interest in physiology and have indicated that they felt each course might be stronger if the alliance were closer. This is a different situation from that of the land-grant colleges where so many of the students in horticulture take botany, chemistry, and physiology, and then go into some commercial branch of agriculture—the practical growing of apples, roses, potatoes, or Christmas trees, or the investigations connected with them.

Apparently teachers keep in mind new aspects of physiology, introduce them into the laboratory if this seems advisable, or at least present them briefly in the lecture. In some cases lack of time compels use of not too complicated and well-tried experiments. Standard experiments seem to be the rule, at least for basic problems. Added to the general assignment, there may be latitude in the choice of one or more of several experiments in certain fields according to interest and ability. The girls who ask for problems in which they do not know the answer have ample opportunity to satisfy their healthy curiosity with the aid of some direction from the teacher.

Since it is impossible to cover the subject of plant physiology thoroughly in one-half year or even a year, the trend is toward inclusion of the major phases so that a student has at least heard of them, and toward emphasis on teaching the *student well* rather than the *subject thoroughly*. By teaching a student to think, we may open her eyes to see more clearly what is going on in the world about her. Years ago they said that our New England farms were exhausted, and now they say that the west is being depleted; but China has survived for forty centuries and more. Agriculture and plant physiology in our country are young, and so it is good for the youth of the nation to recognize such problems and to plan well in advance. Agriculture is a basic industry, and the growing of plants may mean the

life or death of a nation; in fact, the growing of plants in America may mean the life or death of this democracy. Lead the student to consider not one isolated process in the laboratory, but to think in terms of the living plant and of a permanent democracy.

DEPARTMENT OF BOTANY
SMITH COLLEGE
NORTHAMPTON, MASSACHUSETTS

NOTES

Richmond Meeting.—The fifteenth annual meeting of the American Society of Plant Physiologists, held at Richmond, Virginia, December 28 to 30, 1938, was made an occasion of quiet but sincere celebration of fifteen years of successful constructive progress. The programs were arranged in parallel sessions, in cooperation with the Physiological Section of the Botanical Society of America, whose officers in the finest spirit agreed to a pooling of papers so that those on a single topic might be arranged in non-conflicting programs. This is the first time that such an arrangement has been found feasible, and it was so successful that we hope this enlightened policy may be followed on future occasions.

The annual dinner on Wednesday evening was featured by the retiring president's address, and by the usual announcements of awards, and election of a corresponding member. Dr. CURTIS'S address, *Education by authority or for authority?* was appreciated as a challenge to educational policy.

Joint sessions were held with the Botanical Society of America, the American Society for Horticultural Science, and the American Phytopathological Society.

On Friday evening a symposium on the teaching of plant physiology was held as the final session, with the leadership of Dr. BURTON E. LIVINGSTON of the Johns Hopkins University. Two formal and two informal addresses made up the program. A large audience remained for the meeting, and it is obvious that earnest thinking is going on in regard to the teaching problems in this field. In response to this obvious interest in the educational aspects of our work, a standing committee of the society has been appointed to give some permanence and opportunity for more consistent planning of the programs. Taken as a whole the entire meeting was well planned. Much credit for the success of the Richmond meeting goes to the officers of the society, and especially to Dr. W. R. ROBBINS, program committee chairman, and his efficient aids, Dr. H. H. ZIMMERLEY, Dr. D. G. CLARK, and Dr. F. P. CULLINAN, *ex-officio* member of the committee.

Hales Award.—The sixth recipient of the STEPHEN HALES award is Dr. JOHN WESLEY SHIVE, who has served for many years as plant physiologist at the New Jersey Agricultural Experiment Station, and professor of plant physiology at Rutgers University. He went to the Station in 1915, and is nearing a quarter of a century of distinguished service in that institution.

Dr. SHIVE was born at Halifax, Pennsylvania, February 13, 1877. His first degrees, Ph.B. and A.M., were obtained at Dickinson College in 1906 and 1907. In 1913 he went to the Johns Hopkins University where he enjoyed a

fellowship for two years, and received his Ph.D. in 1915. During this period he worked on the salt nutrition of plants, out of which work came the three-salt nutrient solutions which, with modifications, have been very widely used among plant physiologists. From Hopkins he went to the New Jersey Station, and has been a great leader in the field of salt requirements of agricultural plants, and general plant nutrition. The citation accompanying the award by Dr. WALTER F. LOEHWING, president of the Society, is as follows:

"In recognition of a life-time of distinguished service to plant physiology, the American Society of Plant Physiologists now confers the STEPHEN HALES Prize for 1938 on JOHN WESLEY SHIVE.

"For nearly twenty-five years associated with the investigation of the mineral requirements of plants; originator of new and of improved methods for the growth of plants in culture under controlled conditions; one of the earliest to appreciate the fundamental relationship of the hydrogen-ion activity of the culture solution to the absorption of the inorganic ions by the plant and to develop practical methods of plant culture based upon these relationships; student of the chemical composition of plant tissues as affected by the nature of the culture solution; inspiring teacher and leader of a devoted corps of younger investigators; a man whom this Society is proud to have among its membership and a scientist whose single-mindedness of purpose and definiteness of aim have led him to achievement of the highest caliber."

Barnes Award.—Each year since 1926 the American Society of Plant Physiologists has elected one or more of its members to a CHARLES REID BARNES life membership in the Society. Every fifth award is made to some distinguished physiologist from foreign lands. The fifteenth award, made at the Richmond meeting, went to Professor Dr. LUDWIG JOST, who for so many years has distinguished the chair of plant physiology at Heidelberg. JOST is not only distinguished for his research. Plant Physiologists all over the world owe him a debt of gratitude for his contributions to the teaching of plant physiology through his text-books, which have been models of excellence. He followed, in this regard, in the footsteps of SACHS and PFEFFER. The award carries with it the good wishes and high regard of American plant physiologists generally.

Corresponding Member.—One corresponding member was elected at the Richmond meeting. This honor was bestowed upon Sir E. JOHN RUSSELL, distinguished director of the Rothamsted Experimental Station, and of the Imperial Bureau of Soil Science, at Harpenden, England. RUSSELL is best known among plant physiologists for his outstanding work, *Soil Conditions and Plant Growth*, the best single work on this subject in the English language. The Society is honored to have him among its group of distinguished corresponding members.

Corporate Seal.—In July 1937, announcement was made at the Denver meeting of the adoption of a design for a corporate seal for the American Society of Plant Physiologists. In fulfillment of an expressed intention to make public the design adopted, we are reproducing it here. The design is readily understood, and needs no explanatory remarks. It emphasizes the

FIG. 1. The corporate seal of the American Society of Plant Physiologists.

practical value of fundamental research dealing with the processes of plant life. The founding of the Society in 1924 was followed by corporate existence in 1932. The adoption of a seal met a government requirement in connection with the revenue laws.

Milwaukee Meeting.—The summer meetings of the American Society of Plant Physiologists have become almost as interesting and valuable as the midwinter meetings, and as well attended. The season is usually appropriate for driving. The next meeting will be held at Milwaukee, Wisconsin, during the week of June 19 to 24, 1939. The chairman of the program committee for this meeting is Dr. W. E. TOTTINGHAM, one of the past presidents of the Society. Working with him are Dean I. L. BALDWIN, and Dr. L. F. GRABER, all of the University of Wisconsin. Announcements will be made at the proper time through the mails, as to the exact dates, hotel arrangements, and the meetings. Excellent highways and railroad connections to Milwaukee will make transportation a simple matter. Milwaukee is famous; its hospitality for these meetings will be appreciated and enjoyed by all who come. Is it unanimous? Excellent intellectual fare is in store for every one.

Program Committee.—In anticipation of the meeting at Columbus in December, 1939, President LOEHWING has appointed Dr. B. S. MEYER, Department of Botany, Ohio State University, Columbus, Ohio as chairman of the program committee for the sixteenth annual meeting. Dr. J. D. SAYRE of the Ohio Agricultural Experiment Station at Wooster has also been appointed to this committee. Those who may be planning to report before the Society at that time may find it advisable to have their titles ready early. The constantly increasing number of papers has made parallel programs advisable, and those who reply most promptly will be most easily accommodated with time. Fuller reports will be made in later numbers of PLANT PHYSIOLOGY.

Patrons.—During the year 1938 two of our members joined the group of patrons of the Society. They are Dr. WALTER F. LOEHWING, of the State University of Iowa, and Dr. HERMAN A. SPOEHR, of the Carnegie Institution of Washington. This increase of patronage is very gratifying, and is an evidence of the deep interest our members are taking in the problems of permanent support for the Society's program. There are now seven patrons. To all of those who have so generously supported the financial needs of the Society we record the deep appreciation of the entire membership. With the progress already made we hope it is not too much to look forward to other additions to the growing list of supporters. The editor once said, and now repeats that there is "no more enduring benefit to mankind which one can leave behind him than the possibility of continuously increasing knowledge." The need for funds to support our program of research and publication was never greater than at the present moment. And the old Scotch proverb that "Mony a mickle makes a muckle" applies to the continuous growth of our funds. We trust that many friends may wish to share in the realization of a permanent financial foundation for the advancement of plant physiology as a science. Plant physiologists have a way of doing the things they really wish to see accomplished.

John Henry Schaffner.—On January 27, 1939, Professor JOHN H. SCHAFFNER, long time professor of botany at Ohio State University, died at Columbus, Ohio. Professor SCHAFFNER was in his seventy-third year, having been born at Agosta, Ohio, July 8, 1866. He received his degrees from Baker (A.B. 1893), and the University of Michigan (A.M. 1894). He was a graduate student at the University of Chicago in 1896–1897, and spent a year at Zürich in 1907–1908. He was professor of natural science at South Dakota preceding his student days at Chicago, joined the faculty of Ohio State University in 1897, and there spent 40 years in teaching and research. For 10 years he was head of the department of botany (1908–

1918). During this period, in 1916, he married CORDELIA GARBER of Mt. Vernon, Ohio. They have three children. Since 1918 he has been Research Professor of Botany at Ohio. He was editor-in-chief of the Ohio Naturalist from 1900 to 1915, and of the Ohio Journal of Science from 1915 to 1917. He was president of the Ohio Academy of Science, 1908–1909, and was a fellow and member of many scientific organizations. He published a number of botanical books, and contributed many papers to science journals. His special field of interest lay in the field of sex and sex reversal among plants. He was specially interested in the cases in which he thought sex was not controlled by chromosome mechanisms.

The science of botany has lost a devoted research man, and the American Society of Plant Physiologists one of its distinguished members. We deeply regret his passing, and extend our sympathy to his bereaved family.

Plant Physiology.—The second edition of Dr. EDWARD C. MILLER'S well known *Plant Physiology* was issued by the McGraw-Hill Book Co. late in 1938. The work has been carefully revised, and many additions made to the material presented in the first edition. It is a monumental volume of 1201 pages. Dr. MILLER has accomplished a task few would be willing to undertake, and he has given us a work which will be of great value for years to come. It is the American equivalent of the great volumes such as SACHS, PFEFFER, and JOST gave to the world. The entire field is covered in 14 chapters, and the author has done remarkably well in summarizing the vast stores of published work. A rough estimate indicates that more than three thousand different authors have been consulted. It is an indispensable volume for both student and instructor, a source of valuable aid in research. Dr. MILLER deserves the thanks of all plant physiologists for his unselfish service, and praise for having accomplished his task in admirable manner. The quoted price of the work is $7.50, and orders may be sent to the McGraw-Hill Book Co., New York.

Plant Physiology.—The second edition of the English translation of N. A. MAXIMOV'S text-book has been published under the title *Plant Physiology;* it is edited by Dr. R. B. HARVEY and Dr. A. E. MURNEEK from a translation of the fifth Russian edition prepared by Dr. IRENE V. KRASSOVSKY. The arrangement has been changed from the earlier edition, and MAXIMOV rewrote much of it because of the great advances made since the earlier text was published. With the additions of the data and viewpoints of American investigators by the editors, it is a new book, and has been given a distinctive title to prevent confusion with the former translation.

The presentation begins with the physico-chemical organization of plants, and their chemical composition and general metabolism. Chapters on res-

piration, and growth, precede those on carbon and nitrogen assimilation. Then the author turns to mineral nutrition, water relations, and translocation of substances within the plant body. The later chapters discuss such topics as resistance to cold and drought, correlations, physiology of development, processes connected with flowering and fruiting, and ripening of fruits and seeds. The final chapter takes up seasonal phenomena such as dormancy and the breaking of dormancy. The sequence is somewhat unusual, and illustrates that various arrangements of a body of facts may be equally acceptable as a guide to study.

This book, popular in its first edition, will probably be even more popular in its second. The situation as regards texts has changed somewhat, as there is now competition between many meritorious works. The Russian plant physiologists have been very active investigators, and Dr. MAXIMOV has been one of the ablest leaders of the Russian school.

It is a boon to American students to have the rich resources of Russian workers made available in this manner. The book contains 473 pages, 144 text figures, and may be obtained from the publishers, the McGraw-Hill Book Co., at $4.50 per copy.

Structure of Economic Plants.—An excellent contribution to the literature of developmental anatomy is this work by Professor HERMAN E. HAYWARD of the Department of Botany, the University of Chicago. The work is divided into two parts, the first of which deals with the general anatomy of plants. The four chapters covering this general field are entitled: cells and tissues and their development; the anatomy of the root; the anatomy of the shoot; and the anatomy of the flower and fruit.

The second part presents detailed studies of the developmental anatomy of certain crop plants. Sixteen different crop plants have been chosen for this exposition, from Gramineae to Compositae. The plants chosen are corn, wheat, onion, hemp, beet, radish, alfalfa, pea, flax, cotton, celery, sweet potato, white potato, tomato, squash, and lettuce. A bibliography is provided for each chapter, and there is a helpful glossary of anatomical terms at the end of the volume. The book is profusely illustrated, 340 figures, and the illustrations are beautifully reproduced. In every way the book is a fine example of book making. The text is written in very clear and readable style, and the material presented will prove extremely valuable to plant physiologists. When anyone works on any kind of plant he should know its anatomy as thoroughly as is exemplified in the treatment of the plants chosen for this text. That is asking a great deal, but not too much, on the anatomical side. The list price of this indispensable work is $4.90 per copy. It is published by the Macmillan Co., New York, to whom orders may be sent.

Physiology of Plants.—Among the new textbooks of Plant Physiology we welcome the appearance of *The Physiology of Plants* by Dr. WILLIAM SEIFRIZ of the University of Pennsylvania. It is a truly elementary work, written simply and clearly, but with enough detail to give the student a wide introduction to the field of plant physiology. There are 26 chapters, and they are relatively brief, the whole work comprising, with index, only 314 pages. The arrangement of subject matter follows a logical order, and the work seems to be very well adapted to the needs of student and teacher. The book is published by John Wiley and Sons, New York, and the price is $3.50 per copy.

General Plant Physiology.—In the July, 1938 number of PLANT PHYSIOLOGY we announced the publication of *General Plant Physiology,* by E. C. BARTON WRIGHT, who is well known to American readers. At the time, it was stated that an American publisher might be announced later. We are happy to call attention to the fact that this work has now been printed in the United States, so that it is readily available for use in our laboratories and class rooms. Orders may be sent to P. Blakiston's Son and Co., Philadelphia. The price for the American printing is $4.50 per copy.

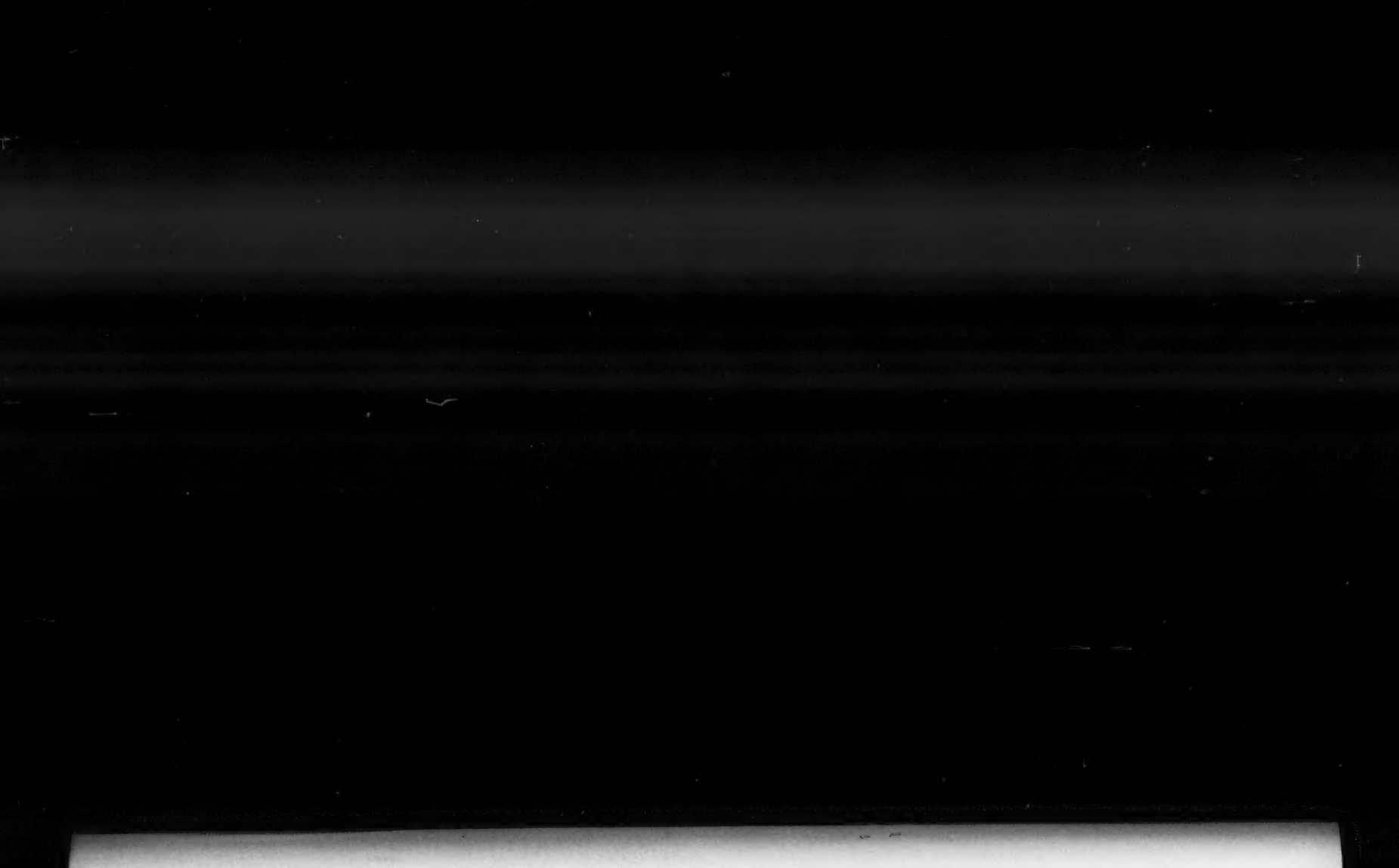
Ascorbic Acid.—An interesting and valuable monograph entitled *L'Acide Ascorbique dans la Cellule et les Tissus* is published as volume 16 of the *Protoplasma Monographien,* a series from the press of Gebrüder Borntraeger, Berlin. The author is A. GIROUD of the faculty of medicine, University of Paris. The material is presented in three parts. The first part is a general introduction to our knowledge of ascorbic acid, and to the various methods of research which have been developed for its detection and quantitative estimation. The second part discusses in detail the facts concerning ascorbic acid in animal cells, tissues, and organs; the third part considers the problems of ascorbic acid in plant cells. The distribution of the acid in various parts of the plant body, variations with age, seeds, and germination, mature plants, physiological considerations, relations to the chlorophyll function, relationship to sugars, and the influence of ascorbic acid on growth. The several bibliographies list about 450 titles. The publication is timely, and brings together a wide spread literature. The quoted price is RM 12 for cloth bound copies. Orders may be addressed to Gebrüder Borntraeger, W 35 Koester Ufer 17, Berlin, Germany.

Advances in the Chemistry of Natural Organic Substances.—The press of Julius Springer, Wien, Germany, has issued the first volume of an annual, *Fortschritte der Chemie organischer Naturstoffe,* which is a collection of summaries in certain fields of plant biochemistry. The editor is L. ZECHMEISTER, Pécs, with the collaboration of A. BUTENANDT, F. KÖGL, W.

N. HAWORTH, and E. SPÄTH. The high standing of these keen investigators is a guarantee that this annual will present an unusually valuable series of reviews. The volume for 1938 contains seven reports. The first deals with the newer methods for glucoside syntheses, prepared by G. ZEMPLÉN. It includes alkylglucoside syntheses by chemical means, biochemical syntheses, syntheses from acetohalogen compounds, transformations of β to α forms, mercury salt methods, preparation of biosides of the α series without the employment of acetohalogen compounds, and phenol-glucoside- or phenol-bioside-syntheses by HELFERICH'S method with the aid of $ZnCl_2$ or p-toluol-sulphonic acid.

The succeeding reviews deal with the following topics: The component glycerides of vegetable fats, by T. P. HILDITCH; recent advances in the chemistry of the sterols, by I. M. HEILBRON and F. S. SPRING; cozymase, by H. VON EULER; nucleic acids, by H. BREDERECK; chlorophyll, by A. STOLL and E. WIEDEMANN; and the application of physical methods to the study of natural substances: form and size of dispersed molecules: roentgenography, by O. KRATKY and H. MARK.

All of them are valuable monographs on the recent work in the fields mentioned. The work is illustrated with 41 text figures, and is provided with extensive author and subject indexes. Every student of the biochemical processes of plants and animals will welcome these authoritative accounts of the recent accomplishments of biochemists. A complete file of this work will be needed in every library, and on the desks of the workers. Orders should be addressed to Julius Springer, Wien (Vienna).

Poisonous Plants.—Under the title *Poisonous Plants of the United States* Dr. W. C. MUENSCHER of Cornell provides an interesting and informative book on the poisonous vascular plants of the United States. About 400 species are included in the discussion, and they are classified according to the families in which they occur (ENGLER system). Sixty-eight families contain toxic members, and 75 well drawn figures will aid in identification of some of the commonest offenders. In the first section some interesting groups are mentioned. There are almost 100 species listed as causing dermatitis, 12 that have photosensitizing effects on animals, 16 that cause hydrocyanic acid poisoning, 18 that are too strongly seleniferous in regions where selenium is abundant in the soil, 28 that cause undesirable flavors in dairy products, and 18 that are liable to produce mechanical lesions, external or internal, because of spines, awns, hairs, or burs. The work has been very ably done, and the book supplies a long-felt need for comprehensive information in this field.

The publishers are the Macmillan Co., 60 Fifth Ave., New York, to whom orders may be sent. The quoted price is $3.50 per copy.